Public Written Comments

Submitted to PCAST

November 19, 2013 - January 27, 2014

As specified in the Federal Register Notice, because PCAST operates under the Federal Advisory Committee Act (FACA), all public comments and/or presentations will be treated as public documents and will be made available for public inspection, including being posted on the PCAST website.

U.S. Technology to Prevent the Global Theft-by-Identity-Fraud

From: [redacted]
Date: Sat, November 23, 2013 1:13 pm
To: pcast@ostp.gov
Cc: "Joshua Lipman"

Time Sensitive, Urgent and Personal for President Obama's Executive Director, PCAST

Dear Ms. Marjory Blumenthal,

The primary benefits derived from utilizing this new technology is preventing much of the $300-400 billion/year taxpayers pay these thieves and for the waste-in-the-chase (i.e., 10% of federal and states budgets) and likewise the $80-90 billion/year consumers pay.

Per the GAO and CBO, the specific *financial sources* for this national outlay of $1 billion/day include Social Security, Medicare, Medicaid, Insurance, Tax Rebates, Credit Cards and SmartPay Cards.

Many theft-prevention attempts have been and are being made by government, industry and academia. None have been able to overcome the pivotal crime-enabling factor: *Removing identity parameters from such financial systems will prevent theft but will also prevent legitimate operations,* the S&T Catch-22.

Fortunately, the addition of the mag-strip to plastic financial cards in the 1950s, the advent of computers and like devices in the 1960s, the World Wide Web and e-commerce Internet technologies in the 1980s finally provided the technological means to overcome this long-standing engineering dilemma.

The U.S. Patent and Trademark Office (USPTO) agreed.

On August 2, 2011 the USPTO granted Utility Patent Chanin No. US 7,991,695 B2 **(*)** Interactive Financial Card System Uniquely Suited For Conducting Financial Transactions On The Internet.

(*) *"B2" - The first application was rejected. The examiners just could not believe the now-obvious solution "is not out there."*

The operating principle which prevents this crime is *personal transaction authorization and liability.*

The pivotal *methodology* is the architecture and protocol of the new and unique electro-mechanical financial card which provides *no personal identity* parameters

and is fully *disposable* because it provides *no financial value* to any but the legitimate card owner.

In addition to the no-value financial card, the other hardware serving this system is integration of an off-the-shelf *card reader* with each involved business and personal computer and like device.

The operating part of the new system is adaptive firmware and software to serve *decentralized security*. (Therefore, decentralization cannot secure verbal financial transactions.)

Today the *primary obstacles* to implement identity-based theft-prevention (a global crime which funds terrorism) are no longer S&T but administrative. A few examples.

Within the U.S. Government:
- Lack of a firm theft-prevention mandate by the POTUS, including that the financial sources, such as the SSA, HHS and IRS, may no longer budget for and may not authorize payments to thieves.
- Therefore, the lack of the needed, overarching technocratic "czar" to integrate cost-effective, theft-prevention methodologies for these "independent" organizations.
- The lack of awareness by federal officials that the acquisition of U.S. granted patents is not governed by FAR 15.605 Unsolicited Proposals but by FAR 52.227-1 Eminent Domain Authorization.
(Every involved federal official is aware of this part of our country's sequester problem and claimed solution.)

For American Industry:
- Few on Wall Street understand the U.S. no longer owns the design of the plastic financial card system.
- Since the 1960s the owners are the International Organization for Standardization (ISO) and International Electrotechnical Council (IEC) wherein the U.S. is a member (American National Standards Institute).
- Their highly detailed standards and specifications (JTC 1/SC-17) are high-value "blueprints" which keep this world-wide criminal enterprise thriving.

Your subject matter response, guidance and help is desperately needed, please. Thank you.

Sincerely,

Harold Chanin
Engineer (ret. DOD)
[redacted]

Re: U.S. Technology to Prevent the Global Theft-by-Identity-Fraud

From: [redacted]
Date: Sat, November 23, 2013 1:45 pm
To: pcast@ostp.gov
Cc: "Joshua Lipman" [redacted]

Corrected.

From: [redacted]
To: pcast@ostp.gov
Cc: "Joshua Lipman" [redacted]
Sent: Saturday, November 23, 2013 1:13:41 PM
Subject: U.S. Technology to Prevent the Global Theft-by-Identity-Fraud

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Sincerely,
Harold Chanin
Engineer (ret. DOD)
[redacted]

Fw: asbmbtoday@asbmb.org, proj015BaB@gmail.com
From: "Devalraju Rambabu"
[redacted]
Date: Mon, November 25, 2013 12:23 am
To: "pcast@ostp.gov" <pcast@ostp.gov> (more)

This is UNDER the *SPECIFIC* REVIEW and ATTENTION OF THE CHIEF EXECUTIVE HIMSELF for this AUGUST MATTER.

OUR ORGANIZATIONS ARE :::

1. **www.rambabu.741.com - - - FOR INTRODUCTION and FRONTAL PAGE DESCRIPTIONS;**
2. **http://drsridhrraocentrmgmt.bloombiz.com/**

(JWALA and Dr. Sridhar Rao Center for Management);

3. **http://www.Medi-e-HEALTHCARE.0catch.com/ [the electronic HEALTH ESTABLISHMENT OF**

(JWALA and Dr. Sridhar Rao Center for Management)

additional e - ADDRESS IN CASE : proj015BaB@gmail.com

On Monday, 25 November 2013 10:35 AM, Devalraju Rambabu [redacted] wrote:

This is UNDER the *SPECIFIC* REVIEW and ATTENTION OF THE CHIEF EXECUTIVE HIMSELF for this AUGUST MATTER.

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From here, PLEASE GO TO "**PROJECT MANAGEMENT**" Link at will [after this ARTICLE]

Restoring Science to It's Rightful Place: Senior Presidential Advisor Reports on Science and Technology Policy in the Obama Administration at Stevens Institute of Technology Lecture Series; [Further edited without any modifications by http://medi-e-healthcare.0catch.com/] - - - proj015BaB@gmail.com and cell : C/o Veeru [Director level Person in Profession] : [INDIA]-9392325629

Dr. John Holdren, Director of the White House Office of Science and Technology Policy, Reviews Efforts to Invest in Science and Technology to Strengthen the Nation.

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5/10/2013 5/10/2013 5/10/2013 5/10/2013

Hoboken, N.J. – That the Obama Administration has "stepped up to support science, technology and innovation" was the overarching message of a speech by Dr. John P. Holdren, Director of the White House Office of Science and Technology Policy (OSTP), at the second installation of the President's Distinguished Lecture Series at Stevens Institute of Technology.

Stevens President Nariman Farvardin called Holdren "one of the world's most influential voices in science and technology" during his introductory remarks in front of a capacity crowd of more than 500, and Holdren responded by delivering a fascinating insider's look at how President Barack Obama has fulfilled his famous first-term inauguration pledge to "restore science to it's rightful place."

Holdren – who earned advanced degrees in aerospace engineering and theoretical plasma physics from Massachusetts Institute of Technology and Stanford University and was previously a professor at Harvard University and University of California, Berkeley – is Obama's senior advisor on virtually all scientific and technical issues that matter to the nation.

The scope of Holdren's office, the OSTP, is incredibly vast. With a staff of 100 and a $5.9 million annual budget, the OSTP is responsible for providing accurate, relevant and timely scientific and technical advice to the President; ensuring executive policies are informed by sound science; and effectively coordinating the scientific and technical work and budgets of dozens of federal departments, offices and agencies.

Through the OSTP, Holdren oversees the National Science & Technology Council (NSTC), including the heads of the NSF, NIH, NASA and NOAA, and dozens of other agencies responsible for information technology R&D, global climate change research, nanotechnology initiatives, emerging technology policy, national oceans policy, and international innovation policy. The OSTP also manages the President's Council of Advisors on Science and Technology (PCAST), of which Holdren is co-chair.

"The place of science in the White House is centered in the OSTP," said Holdren.

Under the Obama Administration, the role of the OSTP has gained significance. Holdren said Obama recognizes that science, technology and innovation are essential to meeting the challenges facing the nation – perhaps more than any previous U.S. President – and is extremely committed to pursuing cutting-edge initiatives that can lead to game-changing innovation in every sector of American society.

Holdren explained how federal support for America's scientific and technological enterprise has increased since Obama took office, as the nation works to "out-innovate, out-educate and out-build the rest of the world." Federal policies – including both new initiatives or resurrected existing initiatives – have called for innovation in healthcare, energy, the environment, education, information technology, security and a wide range of other areas that are critical to improving U.S. competitiveness and national welfare.

"Many of the activities of the OSTP didn't exist or were dormant prior to the Obama Administration," Holdren said.

In healthcare, the Obama Administration – spearheaded by recommendations of the OSTP – has supported the adoption of electronic medical records, worked to improve influenza-vaccine manufacturing, and supported embryonic stem cell and other promising approaches to treat disease. The goal is to both lower costs in healthcare and enable Americans to lead longer and healthier lives.

In energy and the environment, the Obama Administration has created policies to protect against the impacts of global climate change, support sustainable development and foster cleaner sources of energy. The Recovery Act of 2009 devoted $80 billion for clean and efficient energy. The Obama Administration has also pushed for fuel economy standards for trucks, encouraged the federal government to use renewable sources of energy, promoted sustainable agriculture and worked to create green jobs.

Advancing the information technology sector is another major priority of the Obama Administration, bringing government transparency, enabling better communication and collaboration, and making America more safe and secure. It launched Data.gov to give greater access to information and services from the U.S. government. The U.S. Ignite program makes broadband construction both faster and cheaper. Obama has also supported "big data" computing and taken steps to protect America against the "cyber threat."

Another priority of the Obama Administration is cultivating the next generation of skilled, educated, science-savvy Americans by boosting education in science, technology, engineering and mathematics (STEM). Policies like "Race to the Top," "Educate to Innovate" and the STEM Master Teacher Corps serve to emphasize innovation in the K-12 STEM curricula and create more effective STEM teachers, with the goal of restoring America to the top of international science and math test scores and producing more college graduates trained in the STEM fields.

Holdren readily admits that not nearly every science and technology policy recommended by the OSTP, the agencies it oversees, or the President himself have been able to be implemented, given budget constraints and lack of Congressional support. While those challenges remain a factor, in Obama's second term, the priorities will remain the same – to encourage smart investments in science and technology to contribute to economic prosperity, public health, environmental quality and national security.

"Science and technology matter critically to the national agenda," Holdren said, which he reiterated in his post-presentation comments to Stevens faculty members and students.

For 143 years, Stevens has operated under the belief that science and technology are central to meeting the most pressing and complex challenges facing America. The university is doing its part to address the needs of the American people by pursuing it's mission to educate leaders in tomorrow's technology-centric environment while contributing to the solution of the most challenging problems of our time.

Launched this year, Stevens' 10-year strategic plan, The Future. Ours to Create., calls for growth in education and research in five critical areas – healthcare and medicine, sustainable energy, financial systems, defense and security, and STEM education. Innovation and progress in these sectors of society will make America healthier, safer and more globally competitive.

The President's Distinguished Lecture Series, which launched in fall 2012, offers unprecedented access to influential scientists, technologists, policymakers, and business executives at technology driven companies who are shaping 21st century society, in direct alignment with Stevens' own mission. The series focuses on important topics in science and technology, the linkages between societal issues and advances in science and technology, and related policy issues.

Holdren's lecture was made possible in part through a gift from Stevens alumnus Dr. William W. Destler '68, the president of Rochester Institute of Technology.

Watch Holdren's lecture in full on the Stevens YouTube channel.

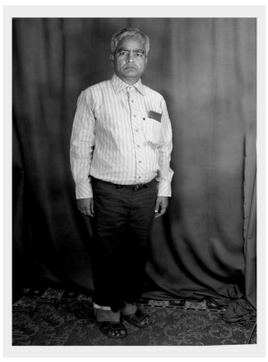
About Stevens Institute of Technology :: Stevens Institute of Technology, The Innovation University®, is a premier, private research university situated in Hoboken, N.J. overlooking the Manhattan skyline. Founded in 1870, technological innovation has been the hallmark and legacy of Stevens' education and research programs for more than 140 years. Within the university's three schools and one college, more than 6,100 undergraduate and graduate students collaborate with more than 350 faculty members in an interdisciplinary, student-centric, entrepreneurial environment to advance the frontiers of science and leverage technology to confront global challenges. Stevens is home to three national research centers of excellence, as well as joint research programs focused on critical industries such as healthcare, energy, finance, defense and STEM education. The university is the fastest-rising college in the U.S. News & World Report ranking of the best national universities, and it is consistently ranked among the nation's elite for return on investment for students, career services programs, and mid-career salaries of alumni. Stevens is in the midst of a 10-year strategic plan, The Future. Ours to Create., designed to further extend the Stevens legacy to create a forward-looking and far-reaching institution with global impact.

- See more at: http://www.stevens.edu/news/content/restoring-science-its-rightful-place-senior-presidential-advisor-reports-science-and#sthash.SKEUf6Y2.dpuf

"PROJECT MANAGEMENT vis a vis Science and Technology"

- PRINCIPAL GOAL : "**smart investments in science and technology**";
- OVER RIDING METHODOLOGY : **in relation to investments in science & technology domain of ACTIVITY, PRIORITIZATION ON THE " PROJECT PHILOSOPHIES, TECHNICS and / or 'Management METHODOLOGIES/COMPUTER SOFTWARE' ";**
- FURTHER MANAGEMENT AND ADMINISTRATION FUNCTIONS TO BE ADOPTED :
  - **Project feasibility studies;**
  - **Project management reviews;**
  - **Project course corrections and REFURBISHMENTS;**
  - **TRACKING and CONTINUOUS MONITERING MACHANISMS;**
  - **INVESTIGATION, REWARD and REPRIMANDS;**
  - **INVESTMENTs [smart] INTO FUNCTIONAL EXECUTIVE as well as NON – EXECUTIVE PRINCIPAL STAFF;**
  - **WORK STANDARDS WITH RESPECT TO SHOP FLOOR WORKERS and WORK SHOP PERSONNEL;**
  - **SEARCH for INNOVATION and TIMELY PROMOTION of 'POLICY SUPPORTIVE' as well as SUBSTANTIALLY GAINFUL INNOVATIONS.**

"Gilliot Bernard, President of ORI" ;;;



DEVALRAJU RAMBABU

The selection of consultants based on the lowest price results in a lower quality of the delivered work. Design costs are generally only a small percentage of the total project cost. Cost savings just on that can lead to "unnecessarily high lifecycle costs later in the project".

" The allocation based on lowest price hinders innovation. The value of a project ultimately depends on the quality of design and expertise of the project."

"As an engineering consultancy company, we face the problem of access from an economic and ecological point of view and try to suggest solutions through innovation. This is the best guarantee for the future and further development of our business."

Projects must be approached from all possible angles.

The current projects require a fully integrated approach in the context of a sustainable concept.

"The engineering consultancy is a privileged partner of the link between investors and developers. It is an inventive and rational approach to providing efficient solutions to achieve the client to achieve. They will have a competitive infrastructure," says Bernard Gilliot. "We must work together with experts from different disciplines to their expertise to integrate and use in our business. With imagination and creativity. The new integrated method in which the engineering firm acts as a translator and an integrator of other scientific branches, and expertise, gives everyone benefits."

THEREFORE, from "Dr. Sridhar Rao Center for MANAGEMENT", ONE innovative 'WORK OF US' WE PRESENT, IS :::

1) INTRODUCTION :: MY PROJECTS SPECIALIZATION 18.11.2011

BAPATLA,
18.11.2011.

- PROJECT FEASIBILITY STUDY & ANALYSIS; - - - WHETHER YOUR IDEAS WORK, ETC...
- PROJECT MANAGEMENT - - - TO PUT YOUR IDEAS TO APPLICATION AND REACH A GOAL.
- PROJECT MANAGEMENT APPRAISAL - - - REVIEW OF PROJECT PROGRESS AT TIMES BY SCIENTIFIC MANAGERIAL METHODS.

I SPECIALIZE IN "FEASIBILITY SCIENCE" APPLICABLE TO PROJECTS GENERALLY OF LARGE AND INVOLVED EFFORT AND/OR INVESTMENT, HTTP://DRSRIDHRRAOCENTRMGMT.BLOOMBIZ.COM AND WWW.RAMBABU.741.COM. I RECOMMEND FEASIBILITY STUDY AND ANALYSIS FOR YOUR PROJECTS IF NOT ALREADY DONE SO BECAUSE THAT PRIMARY STEP IN THE OVERALL PROCESS ALSO BRINGS FORTH MANY DIVIDENDS FOR AND THROUGHOUT THE ENTIRE PROCESS OF CONCEPT TO COMMISSIONING. MY KNOWLEDGE OF ENGINEERING PROJECTS IS STRONG ENOUGH THAT WITH CERTAIN BACKGROUND IN INFORMATION TECHNOLOGY, I AM ALREADY PROCEEDING AHEAD AMIDST OF VARIOUS SUCCESS AFTER SUCCESS SELF CONCEIVED CONCEPT TO COMMISSIONING PROJECTS. I AM CONTINUOUSLY REFINING MY PROJECTS MANAGEMENT AND MANAGEMENT APPRAISAL KNOWLEDGE AND SKILLS.

I REMAIN,

SINCERELY YOURS,

(DEVALRAJU RAMBABU)

2) "A new interpretation and content to "(Project) Feasibility Study" has been arrived at in the Complex ever Science & Technology Domain in this Computer and IT era. The interpretation is equally applicable to all our endeavors including in the Information Technology Domain and the Social and Business Domains."

The Globalized Economic world all around us has given importance to "Projects Concept" to any thing of significance to us and for a viable success in our ventures and endeavors.

By certain Process of intricate research, referencing and by the help of the Providence, we have arrived at a COMPREHENSIVE FEASIBILITY FRAMEWORK for Projects.

"Project Comprehensive Feasibility Framework Study, Analysis and Report Preparation Service for Business, Science & Technology and Information Technology Domains"

3) FROM POINT 2), it led me [rambabu] to : "Feasibility Science" :::

Project Management is a SPECIALTY AREA of work. It PERVADES over all DISCIPLINES OF WORK; especially those of ENGINEERS. We, at www.rambabu.741.com have done a SORT of HIGH LEVEL REVIEW and RESEARCH and HAVE IDENTIFIED GAPS IN THE PRESENT DAY KNOWLEDGE even ACADEMICALLY SPEAKING. IN A COUNTRY LIKE INDIA, THE RESERVE BANK OF INDIA GUIDELINES MAY BE INSISTED UPON BY THE GOVERNMENTS IN POWER IN SUBMITTING A PROJECT PROPOSAL FOR BANK LOANS; But the REALITY OF TODAY's multinational, multidisciplinary and/or Multi Billion DOLLAR PROJECTS etc… is a CAUSE of NIGHTMARES for CONCERNED CHIEF EXECUTIVES IN CONCEPT to COMMISSIONING. This is where WE STAND FIRM WITH OUR "PROPRIETARY DEVELOPMENT" of 'feasibility science'..

THE RESPECTIVE PROJECT DOCUMENTS (like PROSPECTUSUS) :: (THESE ARE NOT PROJECT and/or MANAGEMENT FEASIBILITY REPORTS, BUR ARE INVESTMENT FEASIBILITY REPORTS (in brief)

1. PRELIMINARY DETAILS REPORT (PDR);

2. REASONABLY DETAILED REPORT (RDR).

(BOTH DOCUMENTS LEAD YOU TO "PROGRESSIVE INVESTMENT DECISIONS".)

PROJECT AND CONSULTANCY THEME OF SMILE Services

PROJECTS FORMULATION PHILOSOPHY AND THEME AT SMILE Services ::

1. **SMILE can formulate it's self generated Projects keeping in view the needs of :**
   a. **Catering to the poor and needy;**
   b. **A Management Perspective with Professional Management Philosophy;**
   c. **Economic growth and employment in the State of yours;**
   d. **bold and positive steps to increase investment, efficiency of resource use and employment and adopt special measures for the weak sections with a view to making the State attain a leading position in SMILE generated Business and Industrial Ventures;**
   e. **including the advances in Information Technology and Scientific Computing right from Project Design Stages;**
   f. **providing the Public a Right to partake in the Scrutiny of Project Documents with comprehensible, relevant and reliable details, knowledge and information, only safeguarding the Project with respect to Investor Protection.**
   g. **The poor have to be assisted through more creative interventions for capacity building;**
   h. **a project management approach in implementation;**
   i. **manpower planning, a priori in the Project to the extent feasible.**

CONSULTANCY THEME AND PHILOSOPHY AT SMILE Services ::

- **SMILE Services strives to be a PARTNER IN PROGRESS RATHER THAN AN INDEPENDENT CONSULTANT and/or ADVISOR; This means, SMILE can work partly on Salary basis with respect to any work assigned to it, though SMILE should be retained it's right to recruit it's own staff for the assigned work;**
- **Part of the income generated thus at SMILE Services will be utilized for the Formulation and Design of Novel Projects for further Implementation in your State.**

For US Economy Growth and Defense

From: "Makoto Yanase" [redacted]
Date: Wed, November 27, 2013 3:06 am
To: pcast@ostp.gov

This is information of huge Assets. I'm try to find exact USA Department or Agency. Already several months past. Because, I have proposal for growth US economy. Political situation are always different also very difficult. Every leader of country are countered a lot of problems. Probably no.1 is Economy. How about Defense? Terrorist, Cyber, Weapons, Lobbying, Narcotics,also Climate Change(Hurricane, Tornado) etc., Except Defense, country will be collapse. Also Economy collapse, automatically Defense will be damage. Economy has always problems. Deflation, Inflation, Credit Collapse, Currency Collapse....then Quantitative Easing....Money value will be collapse. Then there are Anglo money, Money Laundering. How can growth GDP? There are so many economy analyst. What is the best way for growth Economy? Since BC, base of economy are value materials. Like SILVER also GOLD. This time GDP are most powerful things. But for my opinion, most safety then always gain value are those materials. Because, money is only paper then value always up and down. Now I want to offer those valuable materials hunted. My offer are huge volumes so I want to discuss Democratic leader country. I believe any country need those commodities for GDP. GDP grow, Defense will be more strong. Please read then assist or advice to me.

Actually, I'm Japanese man then here is Philippines. This is proposal for growth US economy. Economy is the engine of every country. Inflation or Deflation, almost country countered those effect. Every country leaders are try to find good solutions. Maybe Innovation, maybe new technology or science. But National budget are limited. Education, Health care, Infrastructure, Innovation also Defense. Really huge money. Especially, peacemaker country(like USA) has more hard situations. This proposal will be for growth US Economy also help those problems. For my opinion, solution is only one. Find huge Capital(budget). This offer is make budget for future(now).

I explain my offer. I can detect huge Assets(commodities). Simple, I want to get those commodities then use for good way(under US government). Those are not private level. So I decided to contact Democratic leader country. (not Communist, Muslim country). I want to request partnership(joint) with US government. This is complex and confidential proposal. Because, my offer involve huge amount of value commodities(not nature, man made) also I believe need strong back up(like DOD). Those commodities are able to find(detect) only me. Because, I developed own Detector then 17 years trained detector actions. I made confidential movement. Those commodities are sure help US economy growth also Innovation, science for future. But I think so hard to believe only by e-mail. I think best is talk face to face. Those commodities are not this country own(origin). Hide and historical commodities. And nobody find except me. I have existing operation, almost final now. Then I have several sites listed. Each site huge value, huge Billion to Trillion US Dollars deposited(my estimate). Problem, I have situation here, also there are time limited to contact USA. This is right information. Several countries are interested. Once other country know and get those commodities. USA will become more huge problem. Even territory, even Military

situations will be effect. Especially, some countries of Asia, almost collapse Economy now. Very hungry for money. Definitely, my 1st priority is USA. Please assist or advice to me. And I', so afraid contact this country inside. It's so risky this country. I can't wait long time. Please response to me immediately. Today, I attached 1 e-mail and 3 kinds of drawings(3 pages each) for verify my proposal. Please read then response to me immediately.

Makoto

Attach1- (1)

RECHARGE ECONOMIC NEW ENGINE

(1) Innovation business……. No final, continue searching.
(performance cost , kinds of risk)

(2) Defense…….. Reduce cost
(risk for USA, also relation countries)

(3) National Security, etc…. (1)Terrorism
(2)Energy
(3)Climate Change
(4)Costs
(1)Medical
(2)Nature(Hurricane, Tornado, etc)
(3)Emergency , etc.

(4) Education
(5) Infrastructure Development
(6) Investments, Manufacturing

FIND CAPTAL

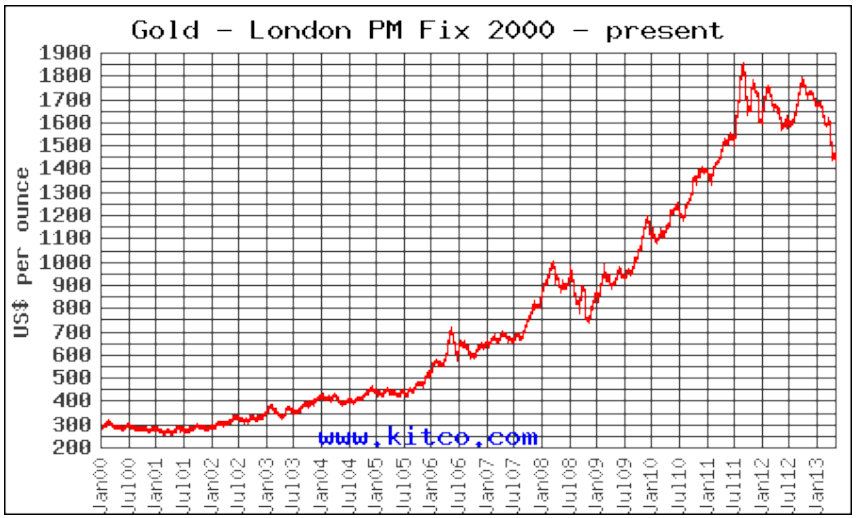
Natural Resources(Best thing)

Attach1- (2)

Economy vs Costs

(1) Defense
(2) Education
(3) Health
(4) Social programs
(5) others

Reduce Costs ?



Attach1- (3)

Find Most Valuable Materials

(1)GOLD value increase every year

(1) 2003 January	356.86	US$ ounce
(2) 2008 January	889.60	US$ ounce
(3) 2013 January	1670.95	US$ ounce

(2)GOLD is based on Economy always.

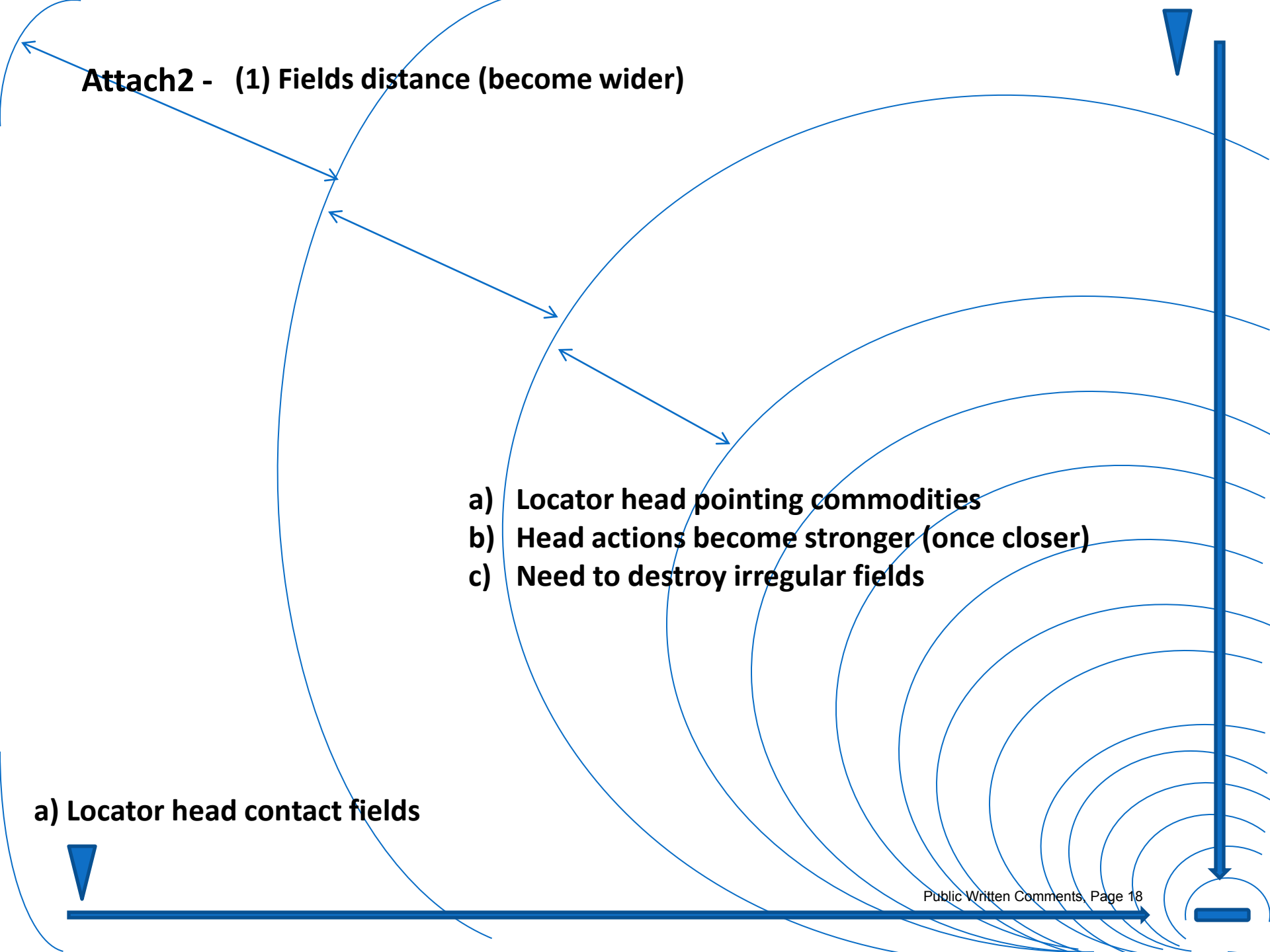
(3)GOLD is the best materials in the world

(Proceed innovation)

OIL, Low cost → **CAPITAL** ← **GOLD**

GOLD is the most Powerful Recharge New Engine

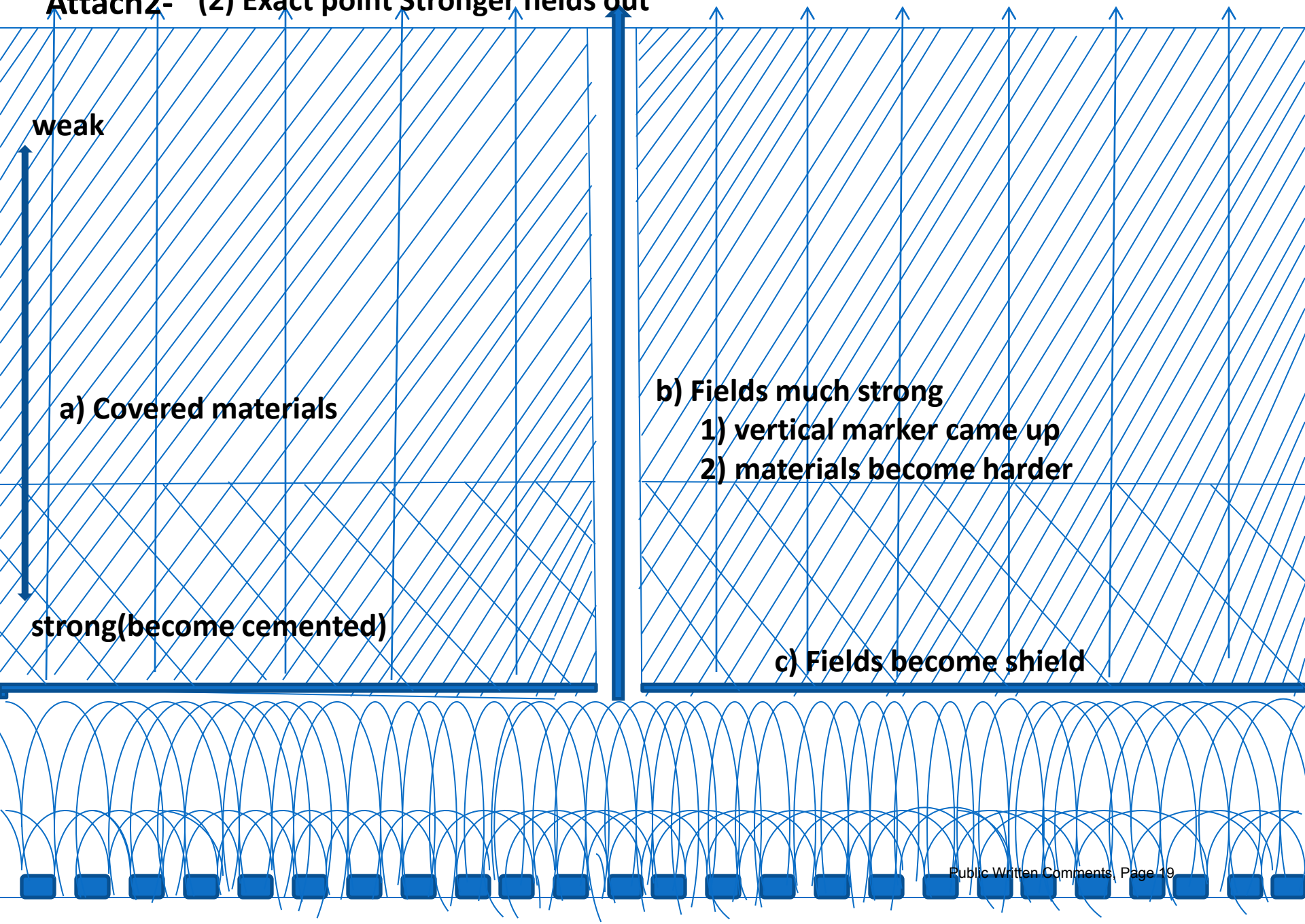
Attach2 - (1) Fields distance (become wider)



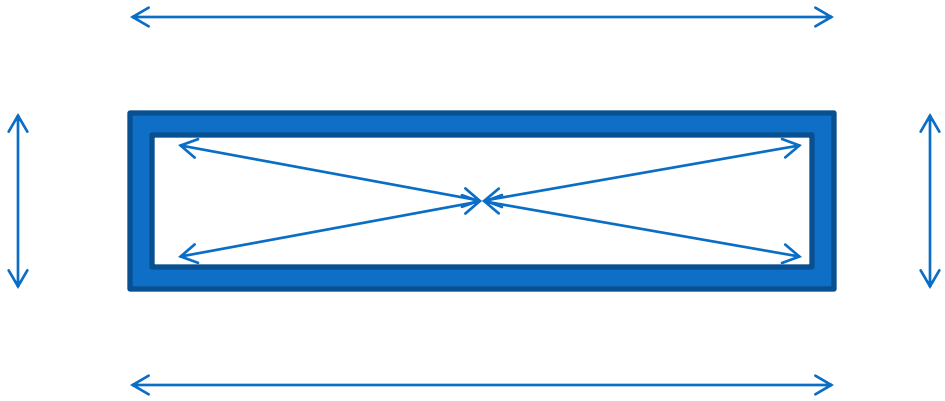
a) Locator head pointing commodities
b) Head actions become stronger (once closer)
c) Need to destroy irregular fields

a) Locator head contact fields

Attach2- (2) Exact point Stronger fields out



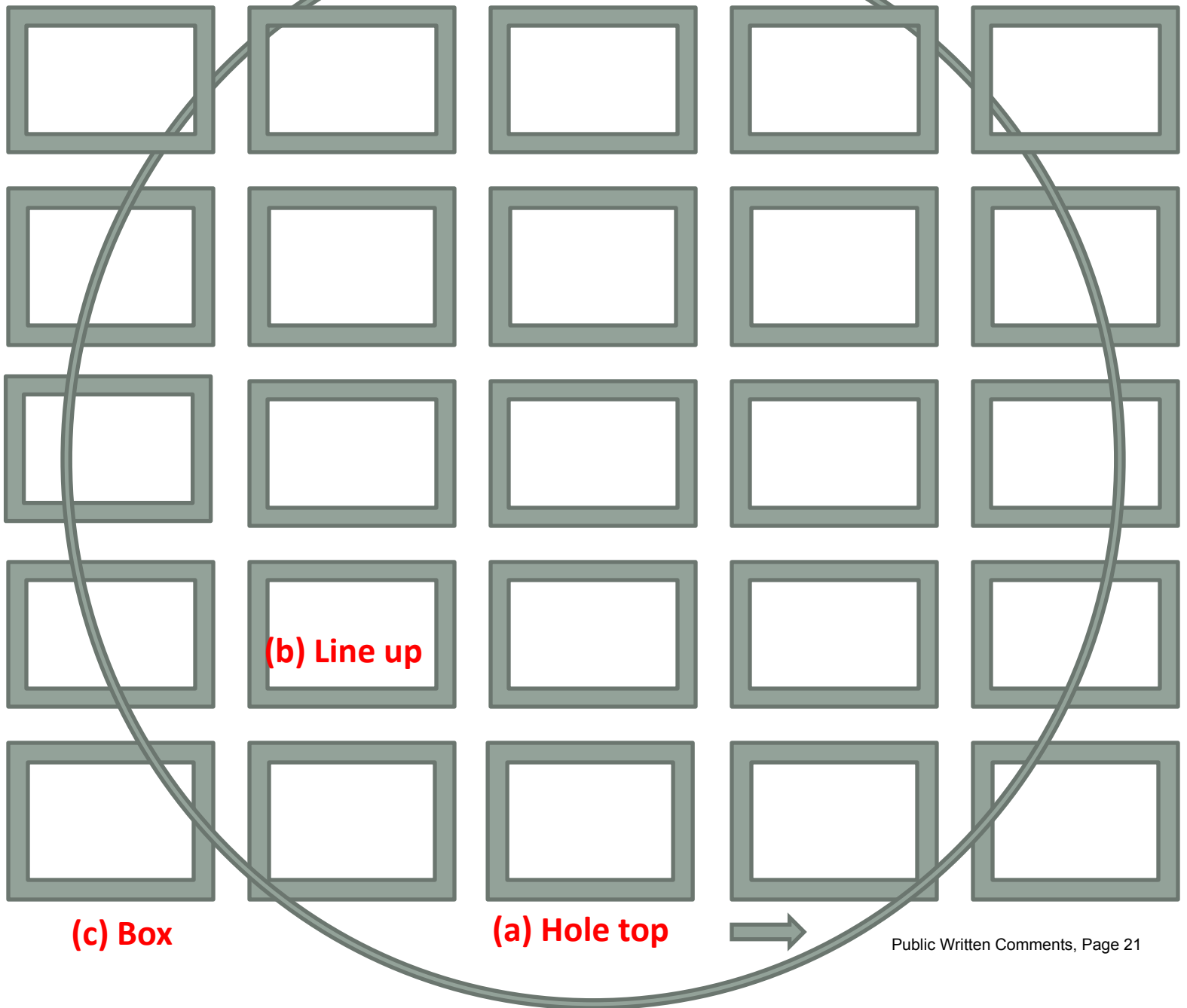
Attach2 - **(3) Locator Action**



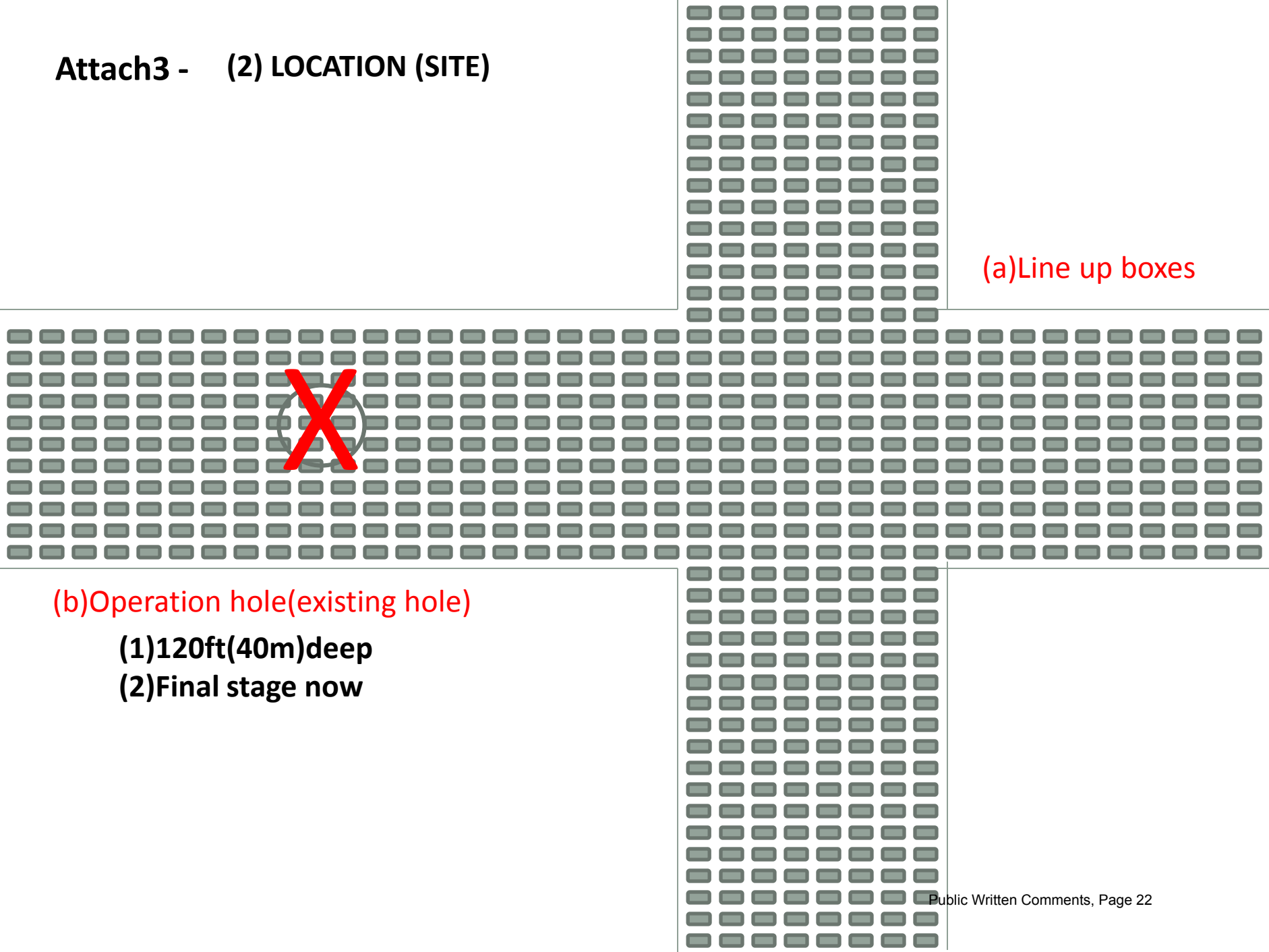
(Gold Bar)

a) **Head cutting size**
b) **Head stop corner and center.**
c) **Head actions become changing**
d) **Head cutting box also line up**
e) **Etc.**

Attach3- (1) SITE VIEW (Operation Hole)



Attach3 - (2) LOCATION (SITE)



(a)Line up boxes

(b)Operation hole(existing hole)

(1)120ft(40m)deep
(2)Final stage now

Attach3 - (3)LOCATION SITE(PICTURES)

(1)Operation house

(2)Operation hole

USA never become position NO.2 every categories. ECONOMY,MILITARY,TECHNOLOGY,SCIENCE..evrything**.** Only 224years, USA always NO.1. World peace depends on USA. World Economy Depends on USA. But now, balance of the world become decline. Especially, Asia some country become monster. Especially military. That's why, it's so risky reduce National budget. For my opinion, money able to buy technology, innovation. Even weapon, even scientist. So money is the base on everything. Find sure **CAPITAL**. That's National Security. Most important for future.

Science, maybe able to save our EARTH. Climate Change(Hurricane, Tornado), Earth Temperature up, Sea level up. Our EARTH is sick now. Which country are able to save? Peoples live in the EARTH, Sick of EARTH become heavy. Because, develop, gas, chemicals.....etc.

Science, maybe able to innovation new energy. Some of Middle East OIL producer country oil stocks bottom alredy. Probably, hard to save that country. Maybe, become war? Someday every producer countries will be empty stocks. How to survive future of Energy? USA is NO.1 OIL consumer country in the world. Probably, new technology(Science) will be save. Innovation save future. Use budget for future of all of the world. **GET CAPITAL**.

This is proposal find CAPITAL for future of our EARTH, also Recharge US economic engine. I produce US government for gain US budget (science, innovation, Recharge Economy, also strong Defense).

---

(1)Target.....Find Most valuable materials in the world. (I'm specialist for find value commodities also special Locator developer). I detect only GOLD BARS(WW2). Those are hide materials, then only Philippines in the world. Those are hard to find this time. Every countries needs long-run economic growth and strong Defense.

(2)Attach(1)-(3) files

(1) **Attach1(3pages)**..... FIND SURE CAPITAL(**for Recharge economic growth USA, etc**)
(1)Deal my technique(know-how) for Detect commodities.
(2)Operation my existing site(final stage now)..UNDER US GOVERNMENT.

(2)**Attach2(3pages)**.....**Functions of gadget(Developed my gadget)**
(1)Detect size of commodities(locator head, cutting GOLD BARS form)
(2)Detect size of box, line up(volumes).
(3)Detect many kinds of magnetic fields, then destroy many irregular fields..
(4)Pointing exact location of deposit. Etc.

(3)**Attach3(3pages)**.....**Existing operation**
(1) Area ..... Rizal province(border of Metro Manila , Philippines)
(2)Conditions.....120FT (40M) deep final stage(confidential operation)
(3)Volume.....5000 TO 10000 TONS (minimum, exactly more)
(4)Value..... 250-500 BUS$ /AREA(MORE) (minimum)
(April10,2013… 1575 US$/troy ounce)
(5)Verify.....Any time able to inspection. There are several area listed already. Need to discussion about that.
ONLY US GOVERNMENT.

I'm Japanese man from Philippines. This offer will be long-run growth US economy also saving for future. I want to deal partnership with US government. This is science, technical skill and abilities. I want to use my potentials and experiences. This country is Philippines. But, origin those commodities are not this country own. Story from WW2. But definitely those value are huge. Surely help long-run economy growth any country. Most important is use for good way. How can clean EARTH except budget?
I trained almost 17years and find all of know-how of mechanism. I want to joint Democratic leader country. So I contact USA. Then I believe US government use for good way. I hate Communist country

also Muslim country in person. Because situation of world now. How about China, North Korea, Middle east. National Budget always increase, hard to reduce. Any countries needs budget. It's so hard to maintain clean EARTH for future. Get huge CAPITAL ,then use for future.

My situation is not easy now. So please move quickly. Please help advice or assist to me. Today, I send with attached. Please check attached ones. Please response to my e-mail, immediately.

Makoto

Please advise the President to Cancel the HHS and IoM MEcfs Redefinition Contract!

From: "Justin Reilly" [redacted]
Date: Mon, December 2, 2013 2:03 am
To: pcast@ostp.gov

Please ask the President to order HHS to adopt the Canadian Consensus Criteria and cancel its contract with the Institute of Medicine (IOM) to redefine ME/CFS

On September 23, thirty-five of the leading ME/CFS researchers and clinicians wrote to HHS Secretary Kathleen Sebelius calling for the Canadian Consensus Criteria (CCC) to be used as the sole case definition for ME/CFS. These experts also urged HHS to abandon its plans to contract with the Institute of Medicine (IOM) to use non-experts to create its own definition.

On the same day, despite an outpouring of patient opposition, HHS announced that it was going forward with the IOM contract to develop its own clinical diagnostic criteria for ME/CFS, instead of adopting the 2003 Canadian Consensus Criteria (CCC) created and endorsed by ME/CFS experts.

Regarding the IOM contract, the thirty-five experts stated, “Since the expert ME/CFS scientific and medical community hads developed and adopted a case definition for research and clinical purposes, this effort (the IOM study) is unnecessary and would waste scarce taxpayer funds that would be much better directed toward funding research on the disease. Worse, this effort threatens to move ME/CFS science backward by engaging non-experts in the development of a case definition for a complex disease about which they are not knowledgeable."

The use of non-experts is especially concerning because, thanks to the bad definitions that HHS has promoted, the disease is so poorly understood that the medical community at large believes the disease is either not real or is a form of depression or deconditioning. ME/CFS is not deconditioning or depression. It is a devastating disease that causes neurological and immunological dysfunction and leaves patients bedridden, housebound and unable to work. ME/CFS costs the U.S. economy an estimated $17-23 billion dollars in lost productivity and direct medical costs.

Given the overwhelming opposition to HHS' plans by both patients and experts, I am asking you to do whatever you can to get HHS to follow the lead of ME/CFS disease experts. HHS must cancel the contract with IOM. HHS must adopt the Canadian Concensus Criteria.

For growth US Economy (Strategy New Cold War)

From: "Makoto Yanase" [redacted]
Date: Tue, December 17, 2013 12:56 am
To: pcast@ostp.gov

This is proposal for help US Economy growth (also Defense)

---

(1) **China Central Bank comment (November 19.2013)**
- (1) China Government appealed change Economy base, bills to **GOLD**.
- (2) China Government holds Federal credit stocks NO.1 in the world.
- (3) China money(GEN) plan become international bills. (same as US Dollars)
- (4) Sale stocks Dollars then buying **GOLD.**

(2) **Border Lines (November 23.2013)**
- (1) China government comment. **China border Line wider**. China use Military power.
- (2) Asia area power balance are changing.
- (3) **Country border Line are moving**. That's also history of China.

---

(1) This is proposal for growth US Economy. I'm specialist for find huge Assets. I produce huge value commodities. Actually, I try to contact US government several months already. It's so hard to contact. I explained several times. My offer commodities are huge value then sure National Security level. So I need to joint strong partner. My 1st priority is USA. Because, Democratic leader country then situation of world this time. (**Economy and New Cold War**)
(2) Economy(GDP) is Engine of country. **Shale gas** are huge engine for USA. Many companies will be back to USA. Asia area are big market especially China. But total Economy renovation needs huge amount of Assets. So I offer my proposal. Sure will be help US economy growth long time. But my choice is USA not China. Because, I believe. USA will be lead mankind for future. Science, Innovation are very important. USA is the leader country most of the time. Then I believe better based on Democratic.
(3) Underground Money, Tax Heaven, Money Laundering. Also Terrorist, Narcotics, Cyber, Nuclear. Then, Education, Health care, Infrastructure, Innovation, Science, Technology. There are so many problems. My proposal will be able to assist.
I can fight for the US FLAG. So I contact USA. My offer will be help US Economy growth. (also Defense) Please check my records mail. Then please assist or advice to me immediately.
(4) Today, I attached **1 mail(guidance) and 3 kinds of drawings**. Please open those one.
Attached as follow.

1	**offer**.....	**Proposal Guidance**	(**2** pages)
2	**attached 1**.....	**Economy and GOLD value**	(**3** pages)
3	**attached 2**.....	**Mechanism of Magnetic Fields**	(**3** pages)
4	**attached 3**.....	**Existing Operation Site**	(**3** pages)

I want to discuss about operation also other listed operations. I want to make confidential operation under US government. I need to discuss face to face your representative person immediately.
Because, each site value **Several Trillion US Dollars** value deposited. This is right information. Please response to me immediately.

Makoto

(P,S) I try to waiting and contact until my time limited. If late, I'll be contact 2nd country. Because , I have no choice.
I'm not playing. I'm not crazy. I'm not SNOWDEN. Just one time try to me FOR USA.

Proposal Guidance

---

1 Defense (Economy and Military)

(1) 224years history in USA. World peace, World Economy are always Depends on USA. But now, balance of the world become decline.

(2) Economy are engine of the country. Inflation or Deflation. Economy are always moving. Increase GDP is the most important for growth Economy. **Shale gas** are good for US economy for future. I want to offer, one of valuable item foreconomy all of the time. That's so value materials. It's **GOLD**.

(3) There are 5 types of Defense area now. Land, Sea, Air, Space and Cyber zone. One of Asia monster country make chaos surround countries. Especially, territories are serious situations now. Of course, Military budget are increase every year. Nuclear weapons, Carrier ship, Long distance missiles. Then Cyber attack.

(4) May 1, 2011, Osama Bin Laden was dead in Pakistan. World famous Terrorist Al-Qaeda came from Pakistan. Pakistan and China had strong connection. Include budget. Question, who is control terrorist? How about North Korea? And Something wrong South Korea this time.

(5) This is proposal for find **Capital(budget)**. This is proposal for hunt huge value materials.(**GOLD BARS**)

2 Innovation(Technology, Science and Energy)

(1) Climate Change(Hurricane, Tornado), Earth Temperature up, Sea level up. Science will be help future. Science leader is USA. Our Earth like sick now. New technology are important for future. But same time come up side effect always. So need to develop medicines for Earth. That's another science. Another budget.

(2) USA found Shale gas now. Those are huge energy for USA. OIL producer countries, especially middle East countries made better lifebecause of Oil money. But like Syria stocks bottom already. Now happened war. Energy are sure life line every country. During World War 2, Japan countered same situations like Syria. Mean, No energy. Most of countries countered kind of problems. Possible to become War. How about China now? China Economy are serious this time. China Military forward outside of countries. I was overlap World War 2.

(3) Money is the power. Capital(budget) are base of GDP. GDP collapse, countries are become risky situations. How can develop new technology, Innovations? Health care, Infrastructure, Innovation, Energy, Education then Defense. There are always endless theme every country.

(1)USA Military world rank…….. No.1
(2)USA IT Broad band speed world rank…….. No.15
(3)USA Infrastructure(2.4% GDP) (1)Bridge…….. 25% of Bride damage or broken
(2)Car accident…. 50% of accident by Road Infra
(22,260 dead/year)
(3)Electric…….. Needs infra
(4)food stamp……. 50million persons(working poor)

3 This is proposal find CAPITAL for Recharge US economic engine.

I want to offer US government for gain US budget(science, innovation, Recharge Economy, also Strong Defense).I offer find Most valuable materials in the world. (I'm specialist for find value Commodities also special Locator developer). I detect only **GOLD BARS(WW2)**. Those are hide and historical materials, then only Philippines in the world. Those are hard to find this time. Those total volumes are so huge. Sure big help US economylong-run growth and strong Defense.

---

4 Attach(1)-(3) files

(1)Attach1(3pages).....**FIND SURE CAPITAL**(**for Recharge economic growth USA, etc**)

(1)Deal my technique(know-how) for Detect commodities.

(2)Operation my existing site(final stage now)..**UNDER US GOVERNMENT**.

---

(2)Attach2(3pages).....**Functions of gadget(Developed my gadget)**

(1)Detect size of commodities(locator head, cutting GOLD BARS form)

(2)Detect size of box, line up(volumes).

(3)Detect many kinds of magnetic fields, then destroy many irregular fields..

(4)Pointing exact location of deposit. Etc.

---

(3)Attach3(3pages).....**Existing operation**

(1) Area ..... Rizal province(border of Metro Manila , Philippines)

(2)Conditions.....**120FT (40M)** deep final stage(confidential operation)

(3)Volume.....**5000 TO 10000 TONS MORE**(minimum)

(4)Value.....**2 TUS$/AREA(MORE)** (minimum)

(January 2,2013.....1693.75US$/troy ounce)

(5)Verify.....Any time able to inspection. There are several arealisted already. Need to discussion about that.

ONLY US GOVERNMENT.

I'm Japanese man from Philippines. This offer will be long-run growth(help) US economy also saving for future. I want to deal partnership with US government. This is science, technical skill and abilities.
I'm specialist of Detect Value commodities. I was Develop for detect those commodities. I developed Special Detector also I got technique(know-how) for find commodities. I want to use my potentials and experiences. This country is Philippines. But those commodities are not this country own(origin). Story from WW2. Hide and historical commodities. Definitely those value are huge. Surely help long-run economy growth any country.

I trained almost 17years and found mechanism of magnetic fields from GOLD BARS. I want to joint Democratic leader country. So I contact USA. Not Communist country also Muslim country. Because situation of world now. How about Asia situations now? National Budget almost increase every year, especially Defense. But GDP Economy are always countered problems most of countries. For my opinion, get huge CAPITAL for that. So I offer this proposal.

My situation is not easy now. So please move quickly. Please help advice or assist to me. Today, I send with attached. Please check attached ones. Please response to my e-mail, immediately. I want to talk your representative immediately.

Makoto

Attach1- (1)

RECHARGE ECONOMIC NEW ENGINE

(1) Innovation business……. No final, continue searching.
(performance cost , kinds of risk)

(2) Defense…….. Reduce cost
(risk for USA, also relation countries)

(3) National Security, etc….
(1)Terrorism
(2)Energy
(3)Climate Change
(4)Costs
(1)Medical
(2)Nature(Hurricane, Tornado, etc)
(3)Emergency , etc.

(4) Education
(5) Infrastructure Development
(6) Investments, Manufacturing

FIND CAPTAL

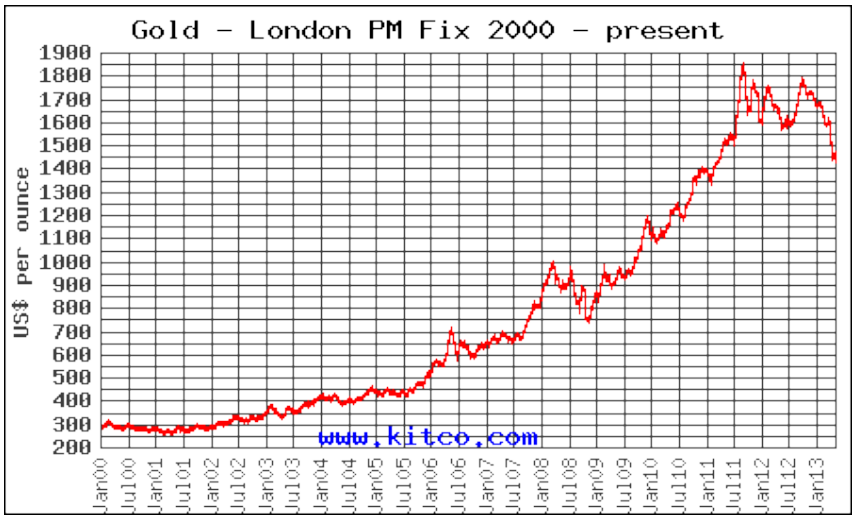
Natural Resources(Best thing)

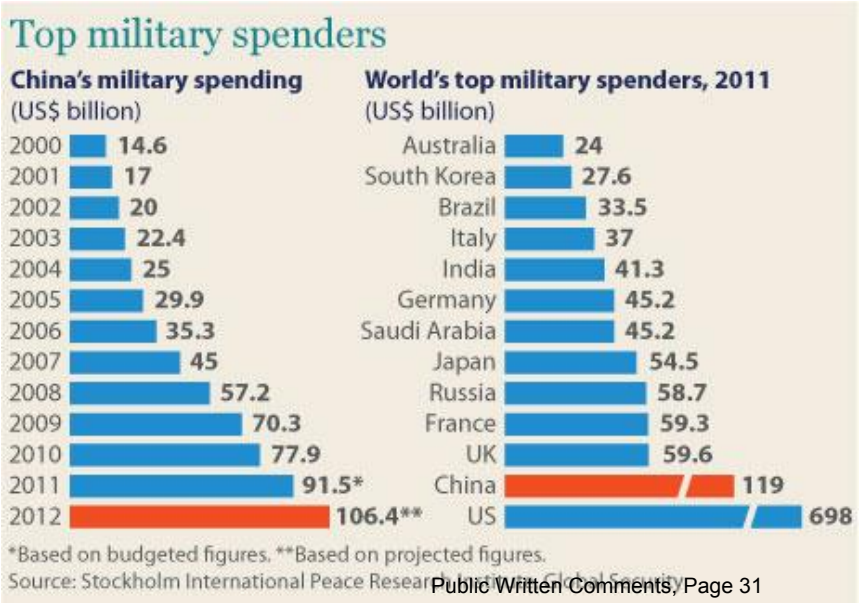
Attach1- (2)

Economy vs Costs

(1) Defense
(2) Education
(3) Health
(4) Social programs
(5) others

Reduce Costs ?





Attach1- (3)

Find Most Valuable Materials

(1)GOLD value increase every year

(1) 2003 January	356.86	US$ ounce
(2) 2008 January	889.60	US$ ounce
(3) 2013 January	1670.95	US$ ounce

(2)GOLD is based on Economy always.

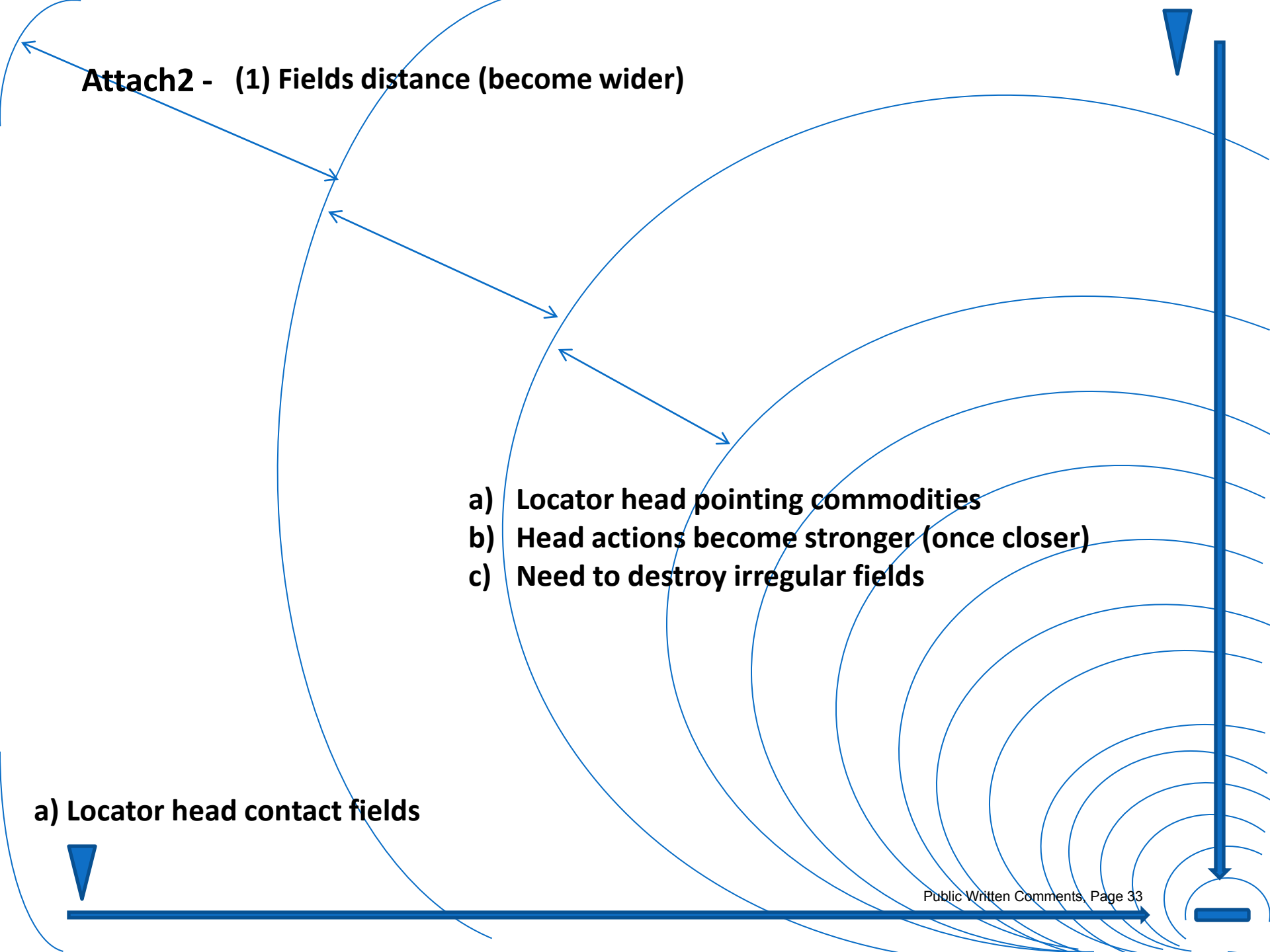
(3)GOLD is the best materials in the world

(Proceed innovation)

OIL, Low cost → **CAPITAL** ← **GOLD**

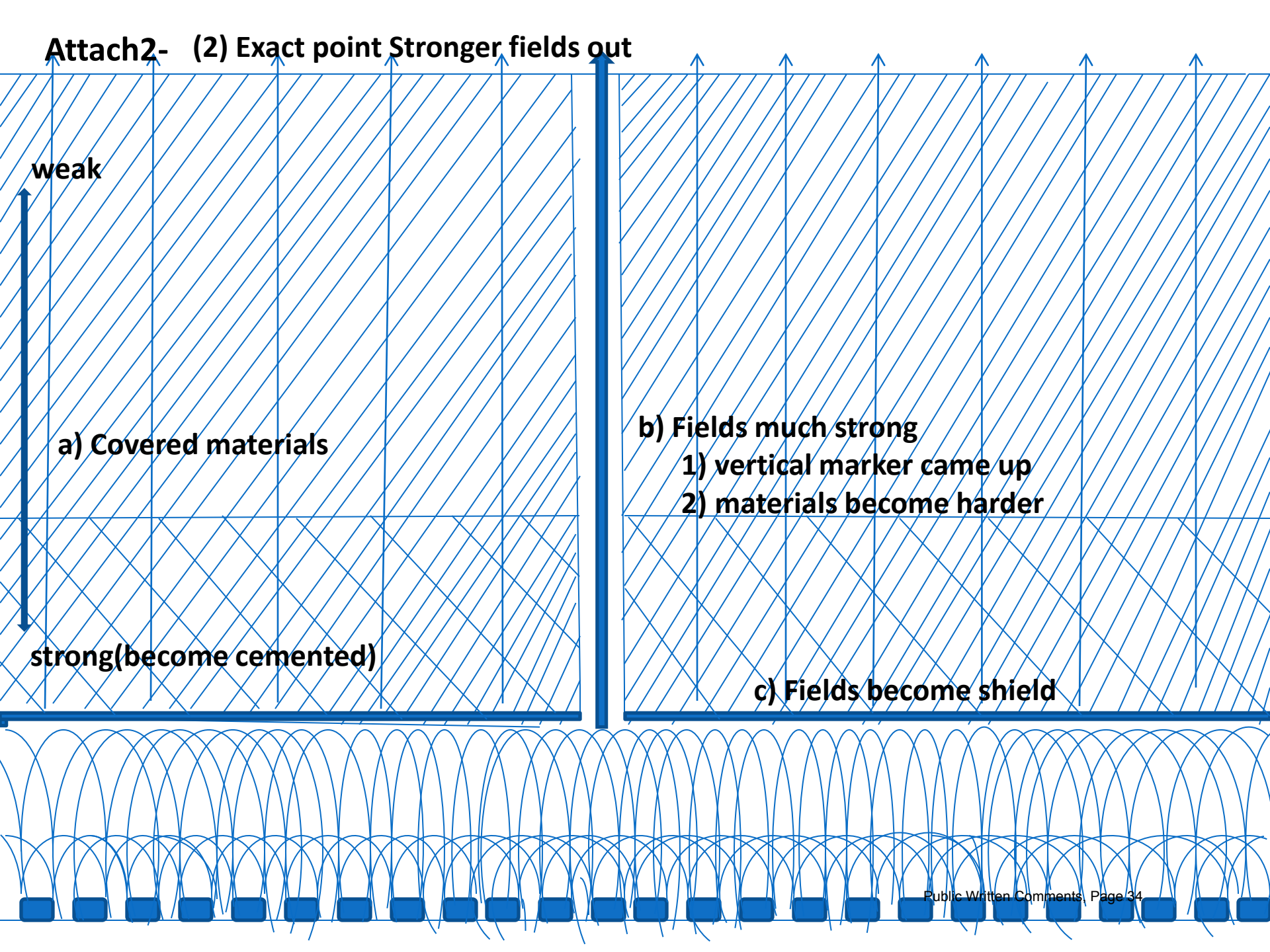
GOLD is the most Powerful Recharge New Engine

Attach2 - (1) Fields distance (become wider)

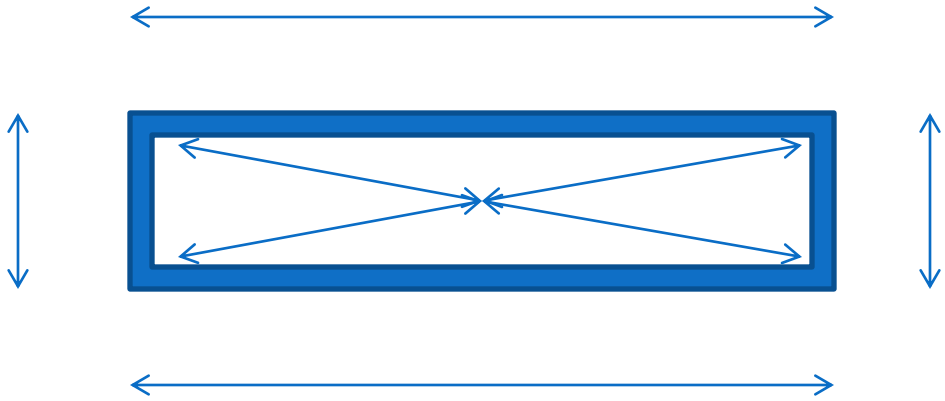


a) Locator head pointing commodities
b) Head actions become stronger (once closer)
c) Need to destroy irregular fields

a) Locator head contact fields


Attach2- (2) Exact point Stronger fields out
weak
a) Covered materials
strong(become cemented)
b) Fields much strong
1) vertical marker came up
2) materials become harder
c) Fields become shield

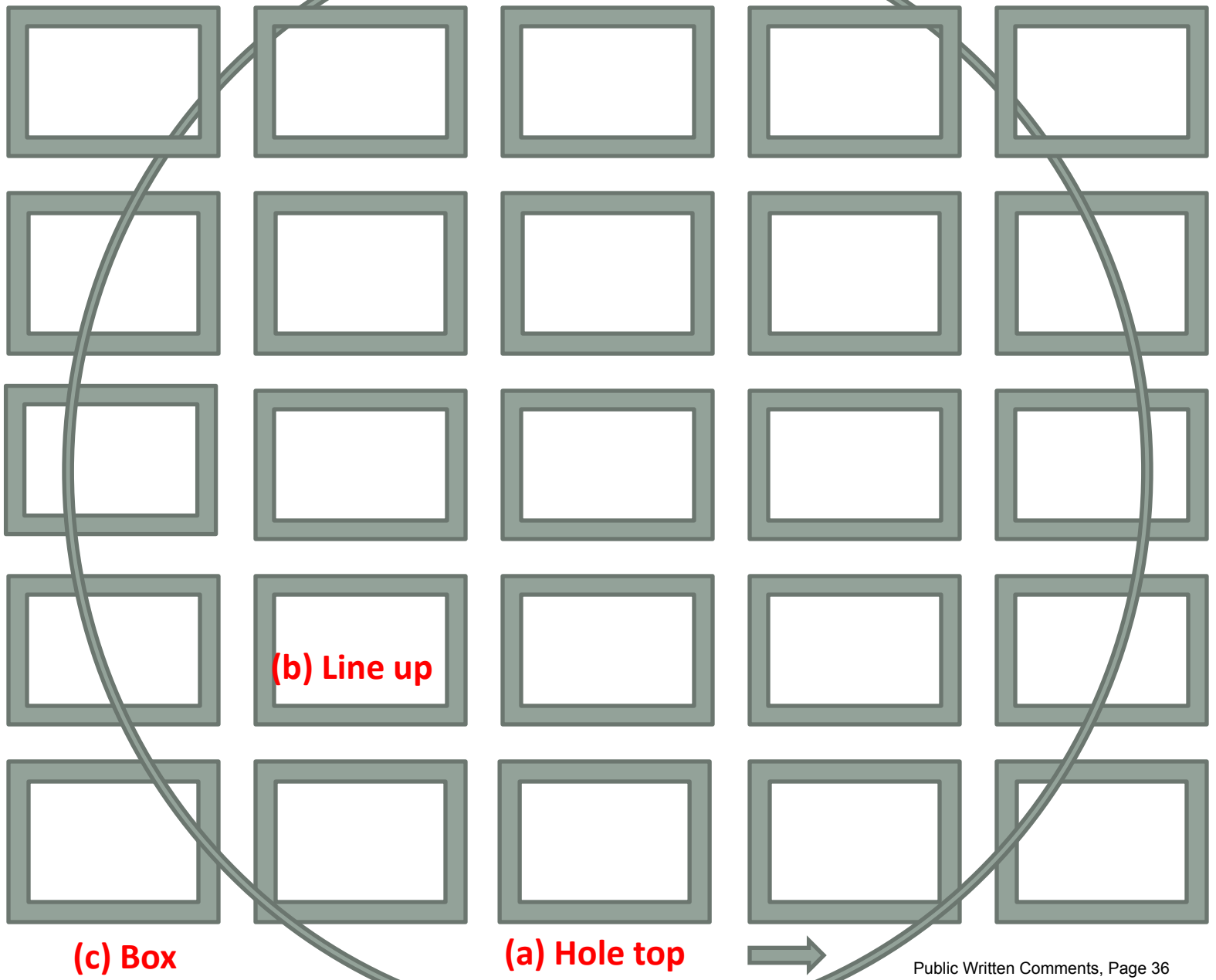
Attach2 - (3) Locator Action



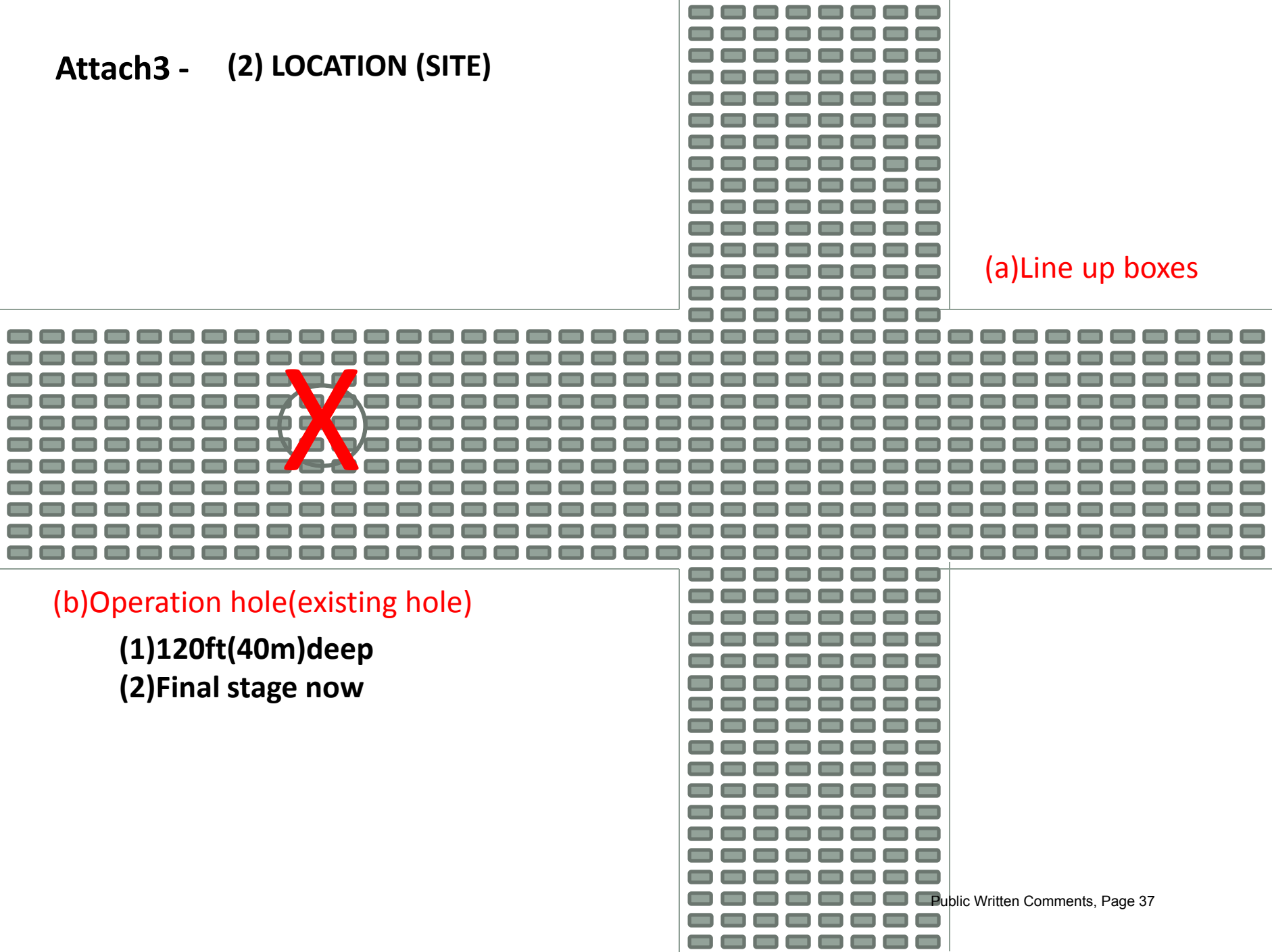
(Gold Bar)

a) Head cutting size
b) Head stop corner and center.
c) Head actions become changing
d) Head cutting box also line up
e) Etc.

Attach3- (1) SITE VIEW (Operation Hole)



Attach3 - (2) LOCATION (SITE)



Attach3 - (3)LOCATION SITE(PICTURES)

(1)Operation house

(2)Operation hole

For US Economy Growth (also Defense)

From: "Makoto Yanase[redacted]
Date: Tue, December 24, 2013 12:29 am
To: pcast@ostp.gov

Dear Mr. Chair

This is **information for help US Economy Growth**. This is involved huge value Assets. **Those Assets will be support US Economy Growth also National Defense, Innovation, Science for future.**

Actually, I sent several times e-mail this address. Today, **I send my letter to Mr. Chair**. I have no direct connection with US government. So I try to contact several Department. I need your assist or advice. I have offer to US government. My offer will be help US Innovation, Science for future. Because there are huge Assets commodities involved. But this is science. I**'m specialist of my offer. I was developed original Detector for find value commodities**. I was explain several times already. So please check my past e-mail. I need your assist or advice.

I want to joint Democratic country. Those Assets are huge volume and very valuable commodities. And historical commodities. So many peoples involved. Many peoples were suffered also many peoples died for those commodities protect.

USA is the leader country of the world. Especially, Defense, Economy are always Engine for the country. I have no idea your position exactly. But for sure, my offer part of science. So I need your suggestions. I believe, **Shale gas are big gain US Economy growth**. That's huge Capital. But **my offer also huge Capital too. Good for Economy Engine. Good for Innovation also Science for future. I want to make partnership with US government.**

This is my general reason. I can contact other country. Even this country inside. But once contact, I will be anti-USA, anti-Democratic. Because, those value are huge. Even USA will be counter huge damage by those commodities. **So I suffer to continue contact USA**. **I don't like become counter part**. **Please contact to Mr. Chair, then asap response to me. I can't manage myself, long time.**

Please open my attached mail. Then as soon as possible response to me.

Thank you

Makoto

Dear Sir.

I'm Makoto Yanase Japanese from Philippines. I have information for help US economy. Please advice or assist to me.

Actually, I sent mail in your e-mail address several times. My information are proposal for huge Assets commodities. Those commodities are huge volume also historical commodities. 1945, world war 2 was finished. During time, Japanese Military bring here huge volume of GOLD bars from Asia countries. General Yamashita was command then hide those one several places. It's so hard to find this time. Because, there are not available exact map also already 68 years past. Of course, some of GOLD were out from area already.

1996, I came here in Philippines. Then I knew history of those GOLD. Then I was starting to look for those commodities. Most important is **know-how of Detect**. Also **exact Detector**. Of course, there are no available kind of gadget. By and by, I developed original Detector. Then I'm starting to training of Detect. I used already 17 years. I spend a lot of times and money. Now I'm perfect for Detect also know-how of operation. Detect is Science. **Need to know GOLD Mechanism also need to find Magnetic fields from GOLD bars**. It's so hard to master Detect skills and so hard kind of experiences. There are nobody in the world, Just only me.

I have existing operation. Final stage this time. Of course, huge volume deposited. Actually, I'm private operator. I'm doing very confidential operation. Reason why, this country situation are very complex and risky. This is not private level anymore. Then I don't need those huge volumes. I'm not Christian. But I believe GOD. Then Japanese soldiers spirits. Because, I countered so many like accidents. But I'm still alive. That's why. By and by, my mind became changing. Better, those commodities use for good way. Not private, for the peoples, for the country. Then, I have listed several huge volume area. So better joint strong party(group), very impossible private. And Democratic country, not communist country not Muslim country. Why not Japan? Because, USA must be manage this country. And there are good reason this time. Like **Territory problems Asia area**. **Air Defense Identification Zone problems**.

I want to operate continue as soon as possible. I lost a lot of things. I lost my life times. But if US government joint together. Dead commodities are get new life.

GOLD are always strong material. Then base on economy long time. **USA got Shale gas**. Economy will be gain. But **totally innovation, science needs sure Capitals**. Those commodities are not this country own(origin). Surrounded countries are also interested those commodities. But it's so hard to find. Especially, there are so many Chinese also Korean this country. Then some of them, try to operation except any analyse. Because, they knew history of commodities. Then those are huge Assets.

Now, I ask help then Joint my proposal.

(1) **I have Existing operation now. Then almost Final situations**. **Of course, those volumes are huge. I can produce my existing site, also inspection anytime**.

(2) **I need to talk your representative person face to face. This is very confidential proposal also complex**.

(3) **I sent proposal before. There are attached 1 e-mail and 3 kinds of drawings(3 pages each). Those are my simple proposal. So please open those attached, then check and response to me.**

Definitely, those commodities are **sure help US Economy Growth, also assist Innovation for future.** I know, there are so many different kind of mails receive every day. But, **this is right information**. Then those commodities are real, also waiting us 68 years for this timing. Then each site huge amount of value deposited. Estimate **Several Trillion US Dollars/site**. World Economy are always Up and Down. **Inflation, Deflation. Currency Collapse, Credit Collapse**. So many issues(problems) countered every country. Especially, USA.

1) **Terrorist, Cyber, weapons, missiles, Nuclear, Narcotics**……
2) **Climate change, Hurricane, Tornado, Tsunami, Earthquake**……..
3) **Science, Innovation, Education, Health care, Infrastructure**……..
4) **Inflation, Deflation, Quantitative Easing**…….
5) **Tax Heaven, Money Laundering, Underground Economy**……
6) **Military(Land, Sea, Air, Space, Cyber)**……

Many problems must be clear by budget**.** **GOLD** are able to erase those problems one by one. I want to use my know-how then support for find commodities. Surely, US Economy will be big help. World are connected everything. By and by, Asia countries also will be better(Economy also Defense). **I can Fight for US FLAG.** Definitely**,** I want to joint only US government(include citizenship)**.** This is my **destiny.** Please support to me. I'm waiting your response. Thank you.

Sincerely

Makoto Yanase

(P.S) Sent Proposal(Before). As follow.

1 offer (proposal guidance)
2 attached 1 (economy)
3 **attached 2** (magnetic fields)
4 **attached 3** **(site drawing)**

Those are my simple proposal. So please check then response to me immediately.

ENDORSING GEOCENTRISM AND REJECTING HELIOCENTRISM BY THE U.S.PRESIDENT.NO RESPONSE FROM THE PCAST OR OSTP OR THE WHITE HOUSE

From: "S.RAJASEKHAFRAN NAIR" <[redacted]>
Date: Sat, January 11, 2014 2:10 am
To: pcast@ostp.gov

THANK YOU.

WHEN WILL I GET THE GRANT AND THE ENDORSEMENT FOR AND ONBEHALF OF MY NEW PRO GOD DISCOVERY ON GECENTRISM BY THE U.S.PRESIDENT?

S.RAJASEKHARAN NAIR

ENDORSING GEOCENTRISM NO REPLY FROM THE WHITE HOUSE SENDING REMINDER

From: "S.RAJASEKHAFRAN NAIR" <[redacted]>
Date: Sat, January 11, 2014 2:10 am
To: pcast@ostp.gov

THANK YOU.

WHEN WILL I GET THE ENDORSEMENT FROM THE U.S.PRESIDENT FOR AND ON BEHALF OF MY NEW PRO GOD DISCOVERY ON GEOCENTRISM?

S.RAJASEKHARAN NAIR

PCAST, the Senate's return of Dr. Cordova's nomination (without a vote); drafting Phillip Sharp at NSF Director

From: "Lloyd Etheredge" <[redacted]>
Date: Tue, January 14, 2014 2:31 pm
To: "Dr. John Holdren - Science Adviser to President Obama and Co-Chair, PCAST" <[redacted]> (more)
Cc: "Dr. Rosina Bierbaum - PCAST" <[redacted]> (more)

Dear PCAST Co-Chairs and Members:

The Senate's return of Dr. Cordova's nomination to President Obama, without a vote, opens the door for the drafting of Phillip Sharp to be the next NSF Director. And perhaps it sends a message? The enclosed letter outlines the case, which I hope will have your support.

A compelling benefit is that Dr. Sharp also has the brilliance to craft a NSF rapid learning system for macro-economics based on the Everything Included Big Data system that his discipline created for cancer and other major diseases. My letter discusses the case that, with his level of scientific leadership at NSF, we could have a very bright future of economic health.

Do you know that the NSF system has removed all economists from the National Science Board and from its senior decision making levels? And, now, from the single token slot that was traditional on its Divisional Advisory Committee? Do you really trust Cordova et al?

Lloyd Etheredge

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[The Policy Sciences Center, Inc. is a public foundation that develops and integrates knowledge and practice to advance human dignity. It was founded by Harold Lasswell, Myres McDougal, and their associates in 1948 in New Haven, CT. Further information about the Policy Sciences Center and its projects, Society, and journal is available at www.policysciences.org.]

THE POLICY SCIENCES CENTER, INC.

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January 14, 2014

Drs. John Holdren, Eric Lander, Maxine Savitz, and William Press, Co-Chairs and Members
President's Council of Advisers on Science and Technology
Eisenhower Executive Office Building
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Dear PCAST Co-Chairs and Members:

The Senate (as you may know) returned to President Obama (without a vote) the nomination of Dr. France Cordova to be the next Director of the National Science Foundation. I urge you to support Dr. Phillip Sharp, the current President of AAAS, as a new nominee by President Obama.

A case against Dr. Cordova (a consensus candidate from an eroding system) is outlined in my testimony (enclosed) to the Senate Committee on Health, Education, Labor, and Pensions. Her National Science Board/NSF system has become a failing experiment. NSF cancelled the guarantee of peer-review Scientific Merit awards, but it did not anticipate the aggressive political pressures and the new and sophisticated mechanisms to carve-up the national science budget (with the rhetoric of "other societal benefits" and "national competitiveness") that were unleashed. There are corrosive perceptions of favoritism and NSF has refused to release audited data and disclose the full set of criteria and weights that are used. In the current Washington climate, professional staff can experience political fear and duress when told to make discretionary decisions. The nation's scientists did not consent to this NSF experiment: anger and alienation are dangerously eroding a system, based on trust, with hundreds of thousands of donated hours to read and write reviews of 40,000 NSF applications/year without a *quid pro quo*.

Only the appointment of an eminent research scientist, like Dr. Phillip Sharp, as NSF Director can restore trust, the guarantee of peer-reviewed Scientific Merit awards, and put NSF back on track.

Dr. Sharp's Appointment: A Bright Future for Economic Growth and Recovery

If Dr. Sharp is appointed, there also is a much brighter future ahead for economic growth and recovery.[1]

Here is the reason: During the years of NSF's lock-down and stonewalling about the catastrophic, layered failures of its Economics program Dr. Sharp's profession (biology, working through NIH)

demonstrated brilliant, trustworthy leadership and remained committed to Scientific Merit decisions. They created large N "Everything Included" databases and built new paradigm-busting machine learning methods for rapid learning and to challenge their conventional wisdom. Once, cancers were classified by the site of occurrence (e.g., breast cancer, lung cancer). Now, biomedical researchers and the medical profession have reconceptualized their understanding of the body and disease. [For example, there may be ten or more types of cancer that occur in the breast or lung (etc.) each with multiple causal pathways and a new universe of possibilities for precision diagnosis and new treatments.] Now, NIH and biomedical researchers are building a transformed global rapid learning system for cancers and (following last month's G-8 summit in London) other major diseases (e.g., Alzheimers and other mental conditions).

The past five years of NIH and biomedical research also could have been NSF and macro-economics! Beginning in 2014, with the leadership of Dr. Phillip Sharp and the solution of NSF's institutional problems, I think it is likely that the scientific community can give the world a macro-economics science for recovery and economic health that matches the attached article by Vogelstein *et al.*

I think that we have a responsibility to correct these breakdowns. The American people and news media have placed great trust in scientists and the self-governance of the scientific community.

Yours truly,

Lloyd Etheredge

Dr. Lloyd S. Etheredge, Project Director

Enclosures:

LSE, Background statement for the Senate Committee on Health, Education, Labor and Pensions. Submitted November 11, 2013.

Bert Vogelstein et al., "Cancer Genome Landscapes," Science, March 29, 2013, pp. 1546-1558.

1. The current NSF/National Science Board system stopped talking about any responsibility for Economics. Faced with growing and legitimate criticism, the NSF system has removed macro-economists from the National Science Board, from all senior NSF decision making, and (now) even from the discipline's single token slot on a large Divisional advisory committee. At a time of national urgency, the appointed Assistant Directors (SBE) have been a specialist in historical demography, and (now) a temporary geneticist. Since the economic crisis began, it has been impossible to have a serious, adult conversation about rapid learning macro-economics with any decision maker at America's NSF with the background and intellectual self-assurance that is required.

Most of the missing variables, needed for a machine-learning Big Data system to connect to reality, appear to be psychological.

November 11, 2013

Statement Concerning the Nomination of France Cordova to be Director of the National Science Foundation

Prepared for the Senate Committee on Health, Education, Labor and Pensions

Chairman Harkin, Ranking Member Alexander and Members: My name is Lloyd Etheredge. I direct the Government Learning Project at the Policy Sciences Center, a public foundation.[1] I have worked for 30+ years to develop the science of rapid learning systems, especially rapid learning by governments. My background includes eight years of teaching research design and data analysis at MIT and serving as Director of Graduate Studies for International Relations at Yale University.

I urge you to reject the nomination of France Cordova to be Director of the National Science Foundation on three grounds.

1.) Dr. Cordova's Stewardship in a National Emergency (2008 -)

Dr. Cordova was appointed to the National Science Board (with accountability to oversee and provide policy guidance to the National Science Foundation) in 2008. She chairs the Committee on Strategy and Budget. NSF has a lead responsibility for basic, interdisciplinary research and transformative ideas in Economics and it administers the core grant for the advisory Committee on National Statistics of the National Research Council, a mechanism that can activate a creative, multi-disciplinary project and strategic planning. Thus, Dr. Cordova's record and candidacy should be evaluated in this light: The leading scientific models of macroeconomics failed catastrophically in 2008. The models still had sufficient truth to prevent another Great Depression but they also have proven, worldwide, to be unreliable scientific guides for rapid recovery and, repeatedly, to mislead policy makers in all countries by their forecasts and promises that their recommended policy options will be effective for a more rapid recovery. Dr. Cordova and NSF have distanced themselves from

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these catastrophic breakdowns and emergency scientific challenges. NSF's senior management team did what the National Science Board wanted them to do: They made themselves invisible, locked-down the NSF social, behavioral and economic sciences (SBE) Directorate, and omitted references to an economic and scientific theory crisis from their strategic plans and budget.

When the space shuttle Challenger exploded, NASA investigated both the scientific and institutional causes and redesigned the shuttle so that it would be safe for future astronauts. By contrast Dr. Cordova - who was trained in astrophysics and who has received awards from NASA - has stonewalled. I know of many letters from serious people and behind-closed-doors pleas for rapid learning and scientific leadership to improve economic ideas, models, and data systems but I am unaware of any document produced by NSF, the NSB, or Dr. Cordova's Committee on Strategy and Budget with serious discussions of breakdowns of scientific Economics. There are no rational plans for rapid learning to collect new data and restore the scientific trustworthiness of NSF-supported theory and research.[2] Dr. Cordova's record of stewardship is chilling.

At this point, let me address two objections that may occur to you: 1.) You may ask: "Is it fair to blame Dr. Cordova? NSF Directors, Acting Directors, and other members of the National Science Board have been silent, too. Surely the lower status people in the NSF system and many economists are more to blame. And Dr. Cordova is not an economist." My suggestion is that you address this question to Dr. Cordova: She is the person who is asking for the public's trust as the new Director of NSF.

You also might ask: 2.) "But does *anyone* know how to improve economic theory and data systems quickly?" For an answer, I suggest that you look at the spectacular rate of learning that has been achieved in this same five year period by competent and honest scientists at a well-run institution (NIH) in the field of cancer research. As the search for new cancer treatments slowed, NIH's senior leadership developed research systems to include new data, expanding to what is now

[2] Leading macro-economists addressed an international IMF summit earlier this year and underscored that, with current theories and data systems, they are out of good ideas to improve the rate of recovery. See Robert J. Samuelson, "The End of Macro Magic," The Washington Post, April 21, 2013. I am not aware of such honesty in National Science Board reports to Congress.

called “Everything” - all variables recorded at the genetic level. And good scientific method - adding a great many new and potentially relevant types of variables - works! Until recently, doctors diagnosed and classified cancers by the site of occurrence: now (with the help of paradigm-busting, machine learning systems that can detect patterns in Big Data systems) we know that there may be 10-15 types of tumors that appear in the breast or lung, each with a different causal pathway. Suddenly an exciting universe of new, precise treatments may be possible for cancer and, perhaps, many other diseases.[3]

Scientists demand integrity and competence. A rational and obvious step for any area of science (especially one as conceptually and sometimes comfortably limited as Economics) is to respond to theories that are not working by searching for missing and potentially relevant variables, building new data systems, and using analysis methods that do not limit you by your preconceptions about reality or causation.

Senators, your work as professional politicians gives you the experience to recognize that rationality is only one part of the story of human behavior. As you may recall, the discipline of economics made a mathematically convenient choice, many years ago, that seemed reasonable at the time, to base its 20th century scientific models and future national policy recommendations on limited psychological ideas and they used variables derived from accounting. However, much more of human psychology can be relevant - e.g., emotional forces, including mistrust, become much more important at times of economic crisis and breakdowns of trust.[4] If your Committee encourages a good choice for NSF Director, many social scientists and other observant people can quickly suggest missing variables and ideas for the new, larger R&D “Everything Included” data systems that may rapidly produce upgraded, new economic theory and accelerate economic recovery.

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[3] Bert Vogelstein *et al.*, “Cancer Genome Landscapes,” in Science, March 29, 2013, pp. 1546 - 1558.

[4] If there is a Kahneman “confidence trap” it is fiercely expensive, and irrelevant, for the Fed to spend billions of dollars on the unproven assumption that the current American problem of slow recovery is a “liquidity trap.” In reality, if the problems of a delayed recovery are “psychological” - as the leading economist at the IMF has suggested - there may be a cornucopia of good options..

2.) The Death of NSF's Honest Broker Role

My second, deep concern is that Dr. Cordova and her associates have overstepped their legal authority as a government agency and knowingly damaged other national institutions. They have killed the Honest Broker role of NSF's social science programs and our nation's research universities without proper notification to your Committee and without public knowledge or legal authority for this historic change.

An example is Governor Romney's claim concerning a dependency syndrome affecting 47% of Americans, undermining their motivation and a willingness to accept responsibility for one's life, and contributing to many economic and social problems. His views echo those of President Reagan. The suppressive record of NSF across the past 30+ years - despite fierce, behind-closed-doors objections within the scientific community - has been pathetic: There are no major social science textbooks with chapters addressing these Republican-believed ideas [and the textbook chapters would need to be rewritten if there were any non-zero coefficients].[5]

The National Science Board (and Dr. Cordova and her associates) were asked to rethink these embarrassing, unwritten, and illegitimate NSF restrictions again, after Governor Romney made it clear that he believed these ideas.

Most Americans, I believe, want public policies that are evidence-based and effective, and they are skeptical that ideologues and loud policy arguers on infotainment television know as much as they believe themselves to know. America deeply deserves an honest and straight-shooting NSF Director with the stature, scientific integrity, and political courage to restore an honorable Honest Broker role for NSF, to challenge aggressive and self-assured people by thoughtful evidence, and who will help to defend the political independence and civic role of our research universities.

[5] I addressed one concern related to this problem in a letter to the Chair and Ranking Member on October 31, 2013. Discussions of other dimensions, written at different historical points and shaped by different periods of frustration, anger, sadness, and hope across 30+ years are online at www.policyscience.net

3.) NSF's Scientific Merit, Peer-Review System: Dr. Cordova and a Pending Meltdown

Senators, many members of this Committee may believe - as most of our nation's scientists still believe - that NSF operates with a guarantee of a Scientific Merit, peer-review decision similar to our jury system. [It is the traditional, trusted system used by other government scientific agencies.] However, this is a misperception and confusion created by subterfuge: Cordova *et al.* have changed the rules and expanded a new system called Merit Review that actually shifts all NSF award decisions to the government's employees (and to themselves). What they call "Merit" introduces long lists of non-Scientific Merit bases for making NSF awards - added new political and social criteria, "too hot to handle" judgments, and unknown weights that they do not fully and equally disclose in advance to all NSF applicants. Nor will Cordova *et al.* disclose audited data to the scientific community showing the real reasons that the government "competitive grant" funds have been awarded or denied. Our nation's research scientists still (partly because they believe the older Scientific Merit system exists and because we believe that we "own" the research system in our fields and are responsible for it) volunteer hundreds of thousands of hours, without compensation, to evaluate about 40,000 applications/year for an NSF and National Science Board that recklessly and offensively have neutralized the older guarantees. This national system is about to melt down.

A contributing problem is money. The so-called Other Benefits rankings, undisclosed program priority weights, higher-level Program Officer decisions, NSB pressures, and other changes have been passionately advocated by lobbyists and interest groups who - behind the public facade of a Scientific Merit, peer-review system and judicial-like integrity - can achieve competitive advantages and carve up growing portions of the national science budget at a time of increased competition for funds. Program Officers are placed under duress to accommodate to these interests, while being absolved from the requirement to keep reliable, complete, and accountable public records. There are chilling rumors that - for example - Texas A&M combined insider information with an aggressive "NSF Days" campus program to secure hundreds of millions of dollars a year when its former President served as Chair of the National Science Board. And that scientific studies of racial prejudice and discrimination against Blacks (along with Honest Broker studies of Republican ideas) are being killed by a fearful and vulnerable bureaucracy.

Possibly, Dr. Cordova and her associates, by continuing to stonewall, will face down the nation's research scientists who want our Scientific Merit peer-review system restored. However, my perception is that a new NSF Director cannot govern with legitimacy unless he or she restores the guarantee of Scientific Merit, peer-review rewards.[6] Otherwise I suspect that an alienated and quiet national meltdown is more likely. The last NSF Director resigned just ahead of a public confrontation and potential No Confidence vote by the AAAS Council, which had angrily discovered NSF's subterfuge and cancellation of traditional guarantees.[7]

Why would you entrust a major national institution to somebody with Dr. Cordova's record of the past five years? My perception is that Dr. Cordova - as an inside and consensus candidate - also lacks the independent stature and support to restore the Scientific Merit, peer-review, guarantee. She may be a candidate that is put forward by an institution that has no intention of changing. And that may be paralyzed in facing the growing self-created problem and outrage of NSF's brutally damaged and conceptually limited Economics research capability; restoring the Honest Broker role of universities in an era of mindlessness; and regaining the trust and loyalty of the nation's scientists.

Concluding Remarks: The Integrity of Democracy and a Pre-Runnymede Breakdown

In conclusion, may I also bring to your attention that Dr. Cordova and the National Science Board have shown poor (and disqualifying) judgment about our system of democratic government? Today, any individual researcher, professional society, or university President who publicly criticizes NSF faces a new top-down NSF system that has dangerously removed guarantees for anonymity,

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[6] Most scientists probably would grant the NSF Director and NSB a 5% Directors Fund for accountable spending of NSF funds for strategic projects or political benefits. NIH also has a Directors Fund.

[7] Many of the interest groups are within the academic world. NSB beneficiaries include aggressive second- and third-tier universities where administrators now openly create "profit Centers" linked to over-charging the national science budget.

Vannevar Bush's vision for a trustworthy NSB/NSF system envisioned leadership by eminent scientists, but positions increasingly are filled by former scientists who have moved-up, permanently, to careers in academic administration. At NIH and the National Cancer Institute (by contrast) more successful and trusted leadership still is available from brilliant, eminent scientists.

removed the right to decisions based on Scientific Merit peer-review of applications, and killed the requirement for full public disclosure, and for independent audit and standards for evaluation and assured fairness and accountability by the higher bureaucracy. If I testify before this Senate Committee and publicly criticize the Chair of the National Science Board for this shift or Dr. Cordova or an Assistant Director for the SBE sciences as irresponsible fools for marginalizing, crippling, and ignoring the NSF Economics program, or for political suppression of Honest Broker evaluation of Republican ideas, or for suppressing studies of racial prejudice, any NSF Program officer will know my identity and that his/her superiors are aware of this public criticism when the Program Officer makes the new discretionary award recommendations over his/her own signatures. (Neither I *nor* the career civil service has the older protections of the Scientific Merit, peer-review system of independent, anonymous evaluation.) The honored model of independent, peer juries and our system of justice, used to design the original NSF system, has been degraded by Cordova *et al.* to a national pre-Runnymede system. The new national scientific management system - crafted by very bad judgment - places everyone under duress and, with a chilling effect, undermines the integrity and freedom of our democratic system. Specifically, it risks a suppressive bias in public testimony and criticisms of NSF and of Dr. Cordova's current candidacy received by your Committee.

How much of a current problem is this duress and bias? At the moment, I suspect that it is small because the dust cloud that has obscured the pre-Runnymede changes in trusted power relationships is just beginning to dissipate. However the dangerous, chilling effects and inhibitions are likely to grow. You should defend the integrity of our democratic political process and appoint an NSF Director with the stature and independence to cancel the power grab and restore the Scientific Merit, independent peer review guarantee. Dr. Cordova is not a candidate who meets these requirements.

Thank you.

Attachment A: Bert Vogelstein *et al.*, "Cancer Genome Landscapes," Science, March 29, 2013, pp. 1546 - 1558.

CANCERGENOMICS
www.sciencemag.org/special/cancergenomics

REVIEW

Cancer Genome Landscapes

Bert Vogelstein, Nickolas Papadopoulos, Victor E. Velculescu, Shibin Zhou, Luis A. Diaz Jr., Kenneth W. Kinzler*

Over the past decade, comprehensive sequencing efforts have revealed the genomic landscapes of common forms of human cancer. For most cancer types, this landscape consists of a small number of "mountains" (genes altered in a high percentage of tumors) and a much larger number of "hills" (genes altered infrequently). To date, these studies have revealed ~140 genes that, when altered by intragenic mutations, can promote or "drive" tumorigenesis. A typical tumor contains two to eight of these "driver gene" mutations; the remaining mutations are passengers that confer no selective growth advantage. Driver genes can be classified into 12 signaling pathways that regulate three core cellular processes: cell fate, cell survival, and genome maintenance. A better understanding of these pathways is one of the most pressing needs in basic cancer research. Even now, however, our knowledge of cancer genomes is sufficient to guide the development of more effective approaches for reducing cancer morbidity and mortality.

Ten years ago, the idea that all of the genes altered in cancer could be identified at base-pair resolution would have seemed like science fiction. Today, such genome-wide analysis, through sequencing of the exome (see Box 1, Glossary, for definitions of terms used in this Review) or of the whole genome, is routine.

The prototypical exomic studies of cancer evaluated ~20 tumors at a cost of >$100,000 per case (*1*–*3*). Today, the cost of this sequencing has been reduced 100-fold, and studies reporting the sequencing of more than 100 tumors of a given type are the norm (table S1A). Although vast amounts of data can now be readily obtained, deciphering this information in meaningful terms is still challenging. Here, we review what has been learned about cancer genomes from these sequencing studies—and, more importantly, what this information has taught us about cancer biology and future cancer management strategies.

How Many Genes Are Subtly Mutated in a Typical Human Cancer?

In common solid tumors such as those derived from the colon, breast, brain, or pancreas, an average of 33 to 66 genes display subtle somatic mutations that would be expected to alter their protein products (Fig. 1A). About 95% of these mutations are single-base substitutions (such as C>G), whereas the remainder are deletions or insertions of one or a few bases (such as CTT>CT) (table S1B). Of the base substitutions, 90.7% result in missense changes, 7.6% result in nonsense changes, and 1.7% result in alterations of splice sites or untranslated regions immediately adjacent to the start and stop codons (table S1B).

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Certain tumor types display many more or many fewer mutations than average (Fig. 1B). Notable among these outliers are melanomas and lung tumors, which contain ~200 nonsynonymous mutations per tumor (table S1C). These larger numbers reflect the involvement of potent mutagens (ultraviolet light and cigarette smoke, respectively) in the pathogenesis of these tumor types. Accordingly, lung cancers from smokers have 10 times as many somatic mutations as those from nonsmokers (*4*). Tumors with defects in DNA repair form another group of outliers (*5*). For example, tumors with mismatch repair defects can harbor thousands of mutations (Fig. 1B), even more than lung tumors or melanomas. Recent studies have shown that high numbers of mutations are also found in tumors with genetic alterations of the proofreading domain of DNA polymerases POLE or POLD1 (*6*, *7*). At the other end of the spectrum, pediatric tumors and leukemias harbor far fewer point mutations: on average, 9.6 per tumor (table S1C). The basis for this observation is considered below.

Mutation Timing

When do these mutations occur? Tumors evolve from benign to malignant lesions by acquiring a series of mutations over time, a process that has been particularly well studied in colorectal tumors (*8*, *9*). The first, or "gatekeeping," mutation provides a selective growth advantage to a normal epithelial cell, allowing it to outgrow the cells that surround it and become a microscopic clone (Fig. 2). Gatekeeping mutations in the colon most often occur in the *APC* gene (*10*). The small adenoma that results from this mutation grows slowly, but a second mutation in another gene, such as *KRAS*, unleashes a second round of clonal growth that allows an expansion of cell number (*9*). The cells with only the *APC* mutation may persist, but their cell numbers are small compared with the cells that have mutations in both genes. This process of mutation followed by clonal expansion continues, with mutations in genes such as *PIK3CA*, *SMAD4*, and *TP53*, eventually generating a malignant tumor that can invade through the underlying basement membrane and metastasize to lymph nodes and distant organs such as the liver (*11*). The mutations that confer a selective growth advantage to the tumor cell are called "driver" mutations. It has been estimated (*12*) that each driver mutation provides only a small selective growth advantage to the cell, on the order of a 0.4% increase in the difference between cell birth and cell death. Over many years, however, this slight increase, compounded once or twice per week, can result in a large mass, containing billions of cells.

The number of mutations in certain tumors of self-renewing tissues is directly correlated with age (*13*). When evaluated through linear regression, this correlation implies that more than half of the somatic mutations identified in these tumors occur during the preneoplastic phase; that is, during the growth of normal cells that continuously replenish gastrointestinal and genitourinary epithelium and other tissues. All of these pre-neoplastic mutations are "passenger" mutations that have no effect on the neoplastic process. This result explains why a colorectal tumor in a 90-year-old patient has nearly twice as many mutations as a morphologically identical colorectal tumor in a 45-year-old patient. This finding also partly explains why advanced brain tumors (glioblastomas) and pancreatic cancers (pancreatic ductal adenocarcinomas) have fewer mutations than colorectal tumors; glial cells of the brain and epithelial cells of the pancreatic ducts do not replicate, unlike the epithelial cells lining the crypts of the colon. Therefore, the gatekeeping mutation in a pancreatic or brain cancer is predicted to occur in a precursor cell that contains many fewer mutations than are present in a colorectal precursor cell. This line of reasoning also helps to explain why pediatric cancers have fewer mutations than adult tumors. Pediatric cancers often occur in non–self-renewing tissues, and those that arise in renewing tissues (such as leukemias) originate from precursor cells that have not renewed themselves as often as in adults. In addition, pediatric tumors, as well as adult leukemias and lymphomas, may require fewer rounds of clonal expansion than adult solid tumors (*8*, *14*). Genome sequencing studies of leukemia patients support the idea that mutations occur as random events in normal precursor cells before these cells acquire an initiating mutation (*15*).

When during tumorigenesis do the remaining somatic mutations occur? Because mutations in tumors occur at predictable and calculable rates (see below), the number of somatic mutations in tumors provides a clock, much like the clock used in evolutionary biology to determine species

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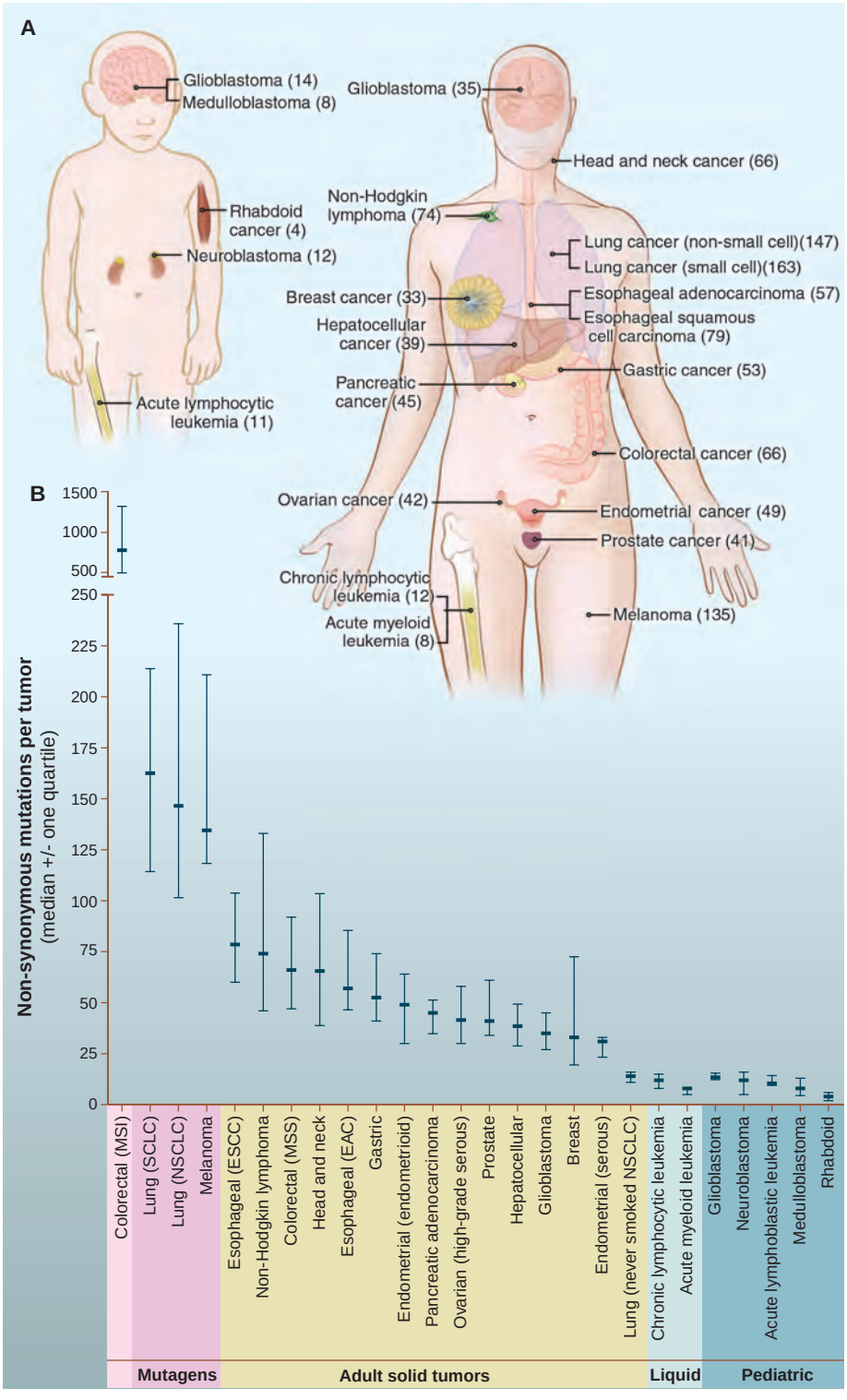

Fig. 1. Number of somatic mutations in representative human cancers, detected by genome-wide sequencing studies. (**A**) The genomes of a diverse group of adult (right) and pediatric (left) cancers have been analyzed. Numbers in parentheses indicate the median number of nonsynonymous mutations per tumor. (**B**) The median number of nonsynonymous mutations per tumor in a variety of tumor types. Horizontal bars indicate the 25 and 75% quartiles. MSI, microsatellite instability; SCLC, small cell lung cancers; NSCLC, non–small cell lung cancers; ESCC, esophageal squamous cell carcinomas; MSS, microsatellite stable; EAC, esophageal adenocarcinomas. The published data on which this figure is based are provided in table S1C.

CREDIT: FIG. 1A, E. COOK

divergence time. The number of mutations has been measured in tumors representing progressive stages of colorectal and pancreatic cancers (*11*, *16*). Applying the evolutionary clock model to these data leads to two unambiguous conclusions: First, it takes decades to develop a full-blown, metastatic cancer. Second, virtually all of the mutations in metastatic lesions were already present in a large number of cells in the primary tumors.

The timing of mutations is relevant to our understanding of metastasis, which is responsible for the death of most patients with cancer. The primary tumor can be surgically removed, but the residual metastatic lesions—often undetectable and widespread—remain and eventually enlarge, compromising the function of the lungs, liver, or other organs. From a genetics perspective, it would seem that there must be mutations that convert a primary cancer to a metastatic one, just as there are mutations that convert a normal cell to a benign tumor, or a benign tumor to a malignant one (Fig. 2). Despite intensive effort, however, consistent genetic alterations that distinguish cancers that metastasize from cancers that have not yet metastasized remain to be identified.

One potential explanation invokes mutations or epigenetic changes that are difficult to identify with current technologies (see section on "dark matter" below). Another explanation is that metastatic lesions have not yet been studied in sufficient detail to identify these genetic alterations, particularly if the mutations are heterogeneous in nature. But another possible explanation is that there are no metastasis genes. A malignant primary tumor can take many years to metastasize, but this process is, in principle, explicable by stochastic processes alone (*17*, *18*). Advanced tumors release millions of cells into the circulation each day, but these cells have short half-lives, and only a miniscule fraction establish metastatic lesions (*19*). Conceivably, these circulating cells may, in a nondeterministic manner, infrequently and randomly lodge in a capillary bed in an organ that provides a favorable microenvironment for growth. The bigger the primary tumor mass, the more likely that this process will occur. In this scenario, the continual evolution of the primary tumor would reflect local selective advantages rather than future selective advantages. The idea that growth at metastatic sites is not dependent on additional genetic alterations is also supported by recent results showing that even normal cells, when placed in suitable environments such as lymph nodes, can grow into organoids, complete with a functioning vasculature (*20*).

Other Types of Genetic Alterations in Tumors

Though the rate of point mutations in tumors is similar to that of normal cells, the rate of chromosomal changes in cancer is elevated (*21*). Therefore, most solid tumors display widespread changes in chromosome number (aneuploidy), as well as deletions, inversions, translocations,

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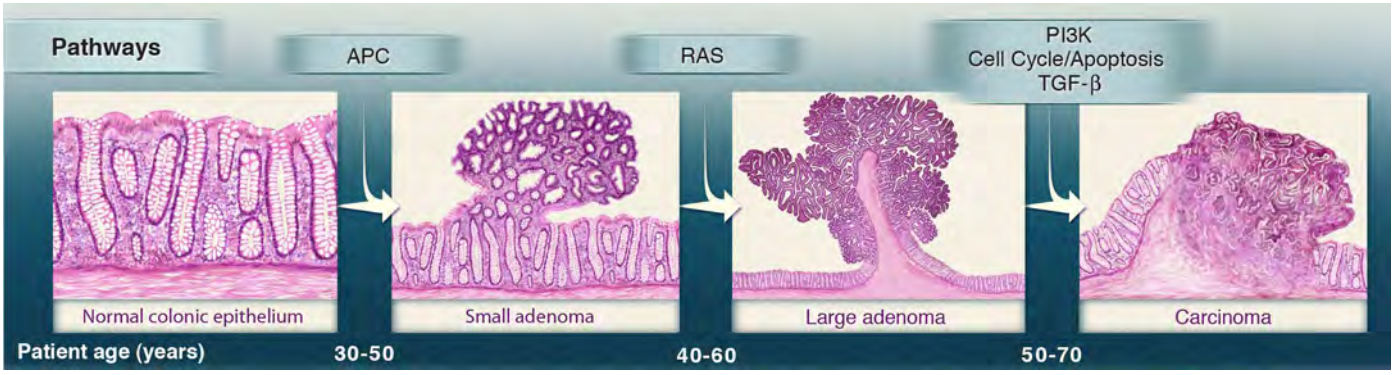

Fig. 2. Genetic alterations and the progression of colorectal cancer. The major signaling pathways that drive tumorigenesis are shown at the transitions between each tumor stage. One of several driver genes that encode components of these pathways can be altered in any individual tumor. Patient age indicates the time intervals during which the driver genes are usually mutated. Note that this model may not apply to all tumor types. TGF-β, transforming growth factor–β.

and other genetic abnormalities. When a large part of a chromosome is duplicated or deleted, it is difficult to identify the specific "target" gene(s) on the chromosome whose gain or loss confers a growth advantage to the tumor cell. Target genes are more easily identified in the case of chromosome translocations, homozygous deletions, and gene amplifications. Translocations generally fuse two genes to create an oncogene (such as *BCR-ABL* in chronic myelogenous leukemia) but, in a small number of cases, can inactivate a tumor suppressor gene by truncating it or separating it from its promoter. Homozygous deletions often involve just one or a few genes, and the target is always a tumor suppressor gene. Amplifications contain an oncogene whose protein product is abnormally active simply because the tumor cell contains 10 to 100 copies of the gene per cell, compared with the two copies present in normal cells.

Most solid tumors have dozens of translocations; however, as with point mutations, the majority of translocations appear to be passengers rather than drivers. The breakpoints of the translocations are often in "gene deserts" devoid of known genes, and many of the translocations and homozygous deletions are adjacent to fragile sites that are prone to breakage. Cancer cells can, perhaps, survive such chromosome breaks more easily than normal cells because they contain mutations that incapacitate genes like *TP53*, which would normally respond to DNA damage by triggering cell death. Studies to date indicate that there are roughly 10 times fewer genes affected by chromosomal changes than by point mutations. Figure 3 shows the types and distribution of genetic alterations that affect protein-coding genes in five representative tumor types. Protein-coding genes account for only ~1.5% of the total genome, and the number of alterations in noncoding regions is proportionately higher than the number affecting coding regions. The vast majority of the alterations in noncoding regions are presumably passengers. These noncoding mutations, as well as the numerous epigenetic changes found in cancers, will be discussed later.

Drivers Versus Passenger Mutations

Though it is easy to define a "driver gene mutation" in physiologic terms (as one conferring a selective growth advantage), it is more difficult to identify which somatic mutations are drivers and which are passengers. Moreover, it is important to point out that there is a fundamental difference between a driver gene and a driver gene mutation. A driver gene is one that contains driver gene mutations. But driver genes may also contain passenger gene mutations. For example, *APC* is a large driver gene, but only those mutations that truncate the encoded protein within its N-terminal 1600 amino acids are driver gene mutations. Missense mutations throughout the gene, as well as protein-truncating mutations in the C-terminal 1200 amino acids, are passenger gene mutations.

Numerous statistical methods to identify driver genes have been described. Some are based on the frequency of mutations in an individual gene compared with the mutation frequency of other genes in the same or related tumors after correction for sequence context and gene size (*22*, *23*). Other methods are based on the predicted effects of mutation on the encoded protein, as inferred from biophysical studies (*24*–*26*). All of these methods are useful for prioritizing genes that are most likely to promote a selective growth advantage when mutated. When the number of mutations in a gene is very high, as with *TP53* or *KRAS*, any reasonable statistic will indicate that the gene is extremely likely to be a driver gene. These highly mutated genes have been termed "mountains" (*1*). Unfortunately, however, genes with more than one, but still relatively few mutations (so called "hills") numerically dominate cancer genome landscapes (*1*). In these cases, methods based on mutation frequency and context alone cannot reliably indicate which genes are drivers, because the background rates of mutation vary so much among different patients and regions of the genome. Recent studies of normal cells have indicated that the rate of mutation varies by more than 100-fold within the genome (*27*). In tumor cells, this variation can be higher and may affect whole

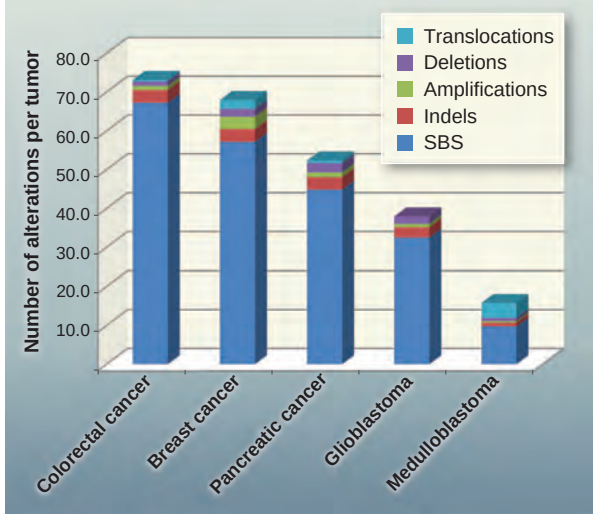

Fig. 3. Total alterations affecting protein-coding genes in selected tumors. Average number and types of genomic alterations per tumor, including single-base substitutions (SBS), small insertions and deletions (indels), amplifications, and homozygous deletions, as determined by genome-wide sequencing studies. For colorectal, breast, and pancreatic ductal cancer, and medulloblastomas, translocations are also included. The published data on which this figure is based are provided in table S1D.

CREDIT: FIG. 2, E. COOK

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Box 1. Glossary

Adenoma: A benign tumor composed of epithelial cells.

Alternative lengthening of telomeres (ALT): A process of maintaining telomeres independent of telomerase, the enzyme normally responsible for telomere replication.

Amplification: A genetic alteration producing a large number of copies of a small segment (less than a few megabases) of the genome.

Angiogenesis: the process of forming vascular conduits, including veins, arteries, and lymphatics.

Benign tumor: An abnormal proliferation of cells driven by at least one mutation in an oncogene or tumor suppressor gene. These cells are not invasive (i.e., they cannot penetrate the basement membrane lining them), which distinguishes them from malignant cells.

Carcinoma: A type of malignant tumor composed of epithelial cells.

Clonal mutation: A mutation that exists in the vast majority of the neoplastic cells within a tumor.

Driver gene mutation (driver): A mutation that directly or indirectly confers a selective growth advantage to the cell in which it occurs.

Driver gene: A gene that contains driver gene mutations (Mut-Driver gene) or is expressed aberrantly in a fashion that confers a selective growth advantage (Epi-Driver gene).

Epi-driver gene: A gene that is expressed aberrantly in cancers in a fashion that confers a selective growth advantage.

Epigenetic: Changes in gene expression or cellular phenotype caused by mechanisms other than changes in the DNA sequence.

Exome: The collection of exons in the human genome. Exome sequencing generally refers to the collection of exons that encode proteins.

Gatekeeper: A gene that, when mutated, initiates tumorigenesis. Examples include *RB*, mutations of which initiate retinoblastomas, and *VHL*, whose mutations initiate renal cell carcinomas.

Germline genome: An individual's genome, as inherited from their parents.

Germline variants: Variations in sequences observed in different individuals. Two randomly chosen individuals differ by ~20,000 genetic variations distributed throughout the exome.

Human leukocyte antigen (HLA): A protein encoded by genes that determine an individual's capacity to respond to specific antigens or reject transplants from other individuals.

Homozygous deletion: Deletion of both copies of a gene segment (the one inherited from the mother, as well as that inherited from the father).

Indel: A mutation due to small insertion or deletion of one or a few nucleotides.

Karyotype: Display of the chromosomes of a cell on a microscopic slide, used to evaluate changes in chromosome number as well as structural alterations of chromosomes.

Kinase: A protein that catalyzes the addition of phosphate groups to other molecules, such as proteins or lipids. These proteins are essential to nearly all signal transduction pathways.

Liquid tumors: Tumors composed of hematopoietic (blood) cells, such as leukemias. Though lymphomas generally form solid masses in lymph nodes, they are often classified as liquid tumors because of their derivation from hematopoietic cells and ability to travel through lymphatics.

Malignant tumor: An abnormal proliferation of cells driven by mutations in oncogenes or tumor suppressor genes that has already invaded their surrounding stroma. It is impossible to distinguish an isolated benign tumor cell from an isolated malignant tumor cell. This distinction can be made only through examination of tissue architecture.

Metastatic tumor: A malignant tumor that has migrated away from its primary site, such as to draining lymph nodes or another organ.

Methylation: Covalent addition of a methyl group to a protein, DNA, or other molecule.

Missense mutation: A single-nucleotide substitution (e.g., C to T) that results in an amino acid substitution (e.g., histidine to arginine).

Mut-driver gene: A gene that contains driver gene mutations.

Nonsense mutation: A single-nucleotide substitution (e.g., C to T) that results in the production of a stop codon.

Nonsynonymous mutation: A mutation that alters the encoded amino acid sequence of a protein. These include missense, nonsense, splice site, translation start, translation stop, and indel mutations.

Oncogene: A gene that, when activated by mutation, increases the selective growth advantage of the cell in which it resides.

Passenger mutation (passenger): A mutation that has no direct or indirect effect on the selective growth advantage of the cell in which it occurred.

Primary tumor: The original tumor at the site where tumor growth was initiated. This can be defined for solid tumors, but not for liquid tumors.

Promoter: A region within or near the gene that helps regulate its expression.

Rearrangement: A mutation that juxtaposes nucleotides that are normally separated, such as those on two different chromosomes.

Selective growth advantage (*s*): The difference between birth and death in a cell population. In normal adult cells in the absence of injury, $s = 0.000000$.

Self-renewing tissues: Tissues whose cells normally repopulate themselves, such as those lining the gastrointestinal or urogenital tracts, as well as blood cells.

Single-base substitution (SBS): A single-nucleotide substitution (e.g., C to T) relative to a reference sequence or, in the case of somatic mutations, relative to the germline genome of the person with a tumor.

Solid tumors: Tumors that form discrete masses, such as carcinomas or sarcomas.

Somatic mutations: Mutations that occur in any non–germ cell of the body after conception, such as those that initiate tumorigenesis.

Splice sites: Small regions of genes that are juxtaposed to the exons and direct exon splicing.

Stem cell: An immortal cell that can repopulate a particular cell type.

Subclonal mutation: A mutation that exists in only a subset of the neoplastic cells within a tumor.

Translocation: A specific type of rearrangement where regions from two nonhomologous chromosomes are joined.

Tumor suppressor gene: A gene that, when inactivated by mutation, increases the selective growth advantage of the cell in which it resides.

Untranslated regions: Regions within the exons at the 5′ and 3′ ends of the gene that do not encode amino acids.

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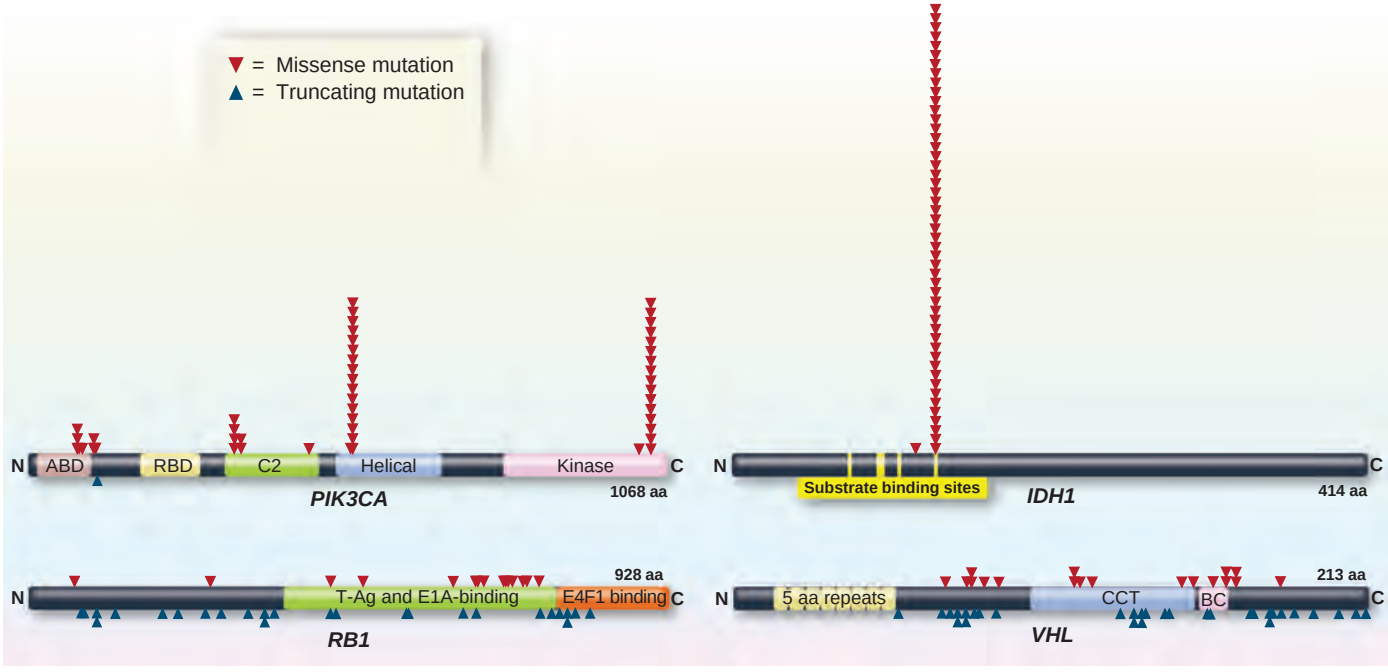

Fig. 4. Distribution of mutations in two oncogenes (*PIK3CA* and *IDH1*) and two tumor suppressor genes (*RB1* and *VHL*). The distribution of missense mutations (red arrowheads) and truncating mutations (blue arrowheads) in representative oncogenes and tumor suppressor genes are shown. The data were collected from genome-wide studies annotated in the COSMIC database (release version 61). For *PIK3CA* and *IDH1*, mutations obtained from the COSMIC database were randomized by the Excel RAND function, and the first 50 are shown. For *RB1* and *VHL*, all mutations recorded in COSMIC are plotted. aa, amino acids.

regions of the genome in an apparently random fashion (*28*). Thus, at best, methods based on mutation frequency can only prioritize genes for further analysis but cannot unambiguously identify driver genes that are mutated at relatively low frequencies.

Further complicating matters, there are two distinct meanings of the term "driver gene" that are used in the cancer literature. The driver-versus-passenger concept was originally used to distinguish mutations that caused a selective growth advantage from those that did not (*29*). According to this definition, a gene that does not harbor driver gene mutations cannot be a driver gene. But many genes that contain few or no driver gene mutations have been labeled driver genes in the literature. These include genes that are overexpressed, underexpressed, or epigenetically altered in tumors, or those that enhance or inhibit some aspect of tumorigenicity when their expression is experimentally manipulated. Though a subset of these genes may indeed play an important role in the neoplastic process, it is confusing to lump them all together as driver genes.

To reconcile the two connotations of driver genes, we suggest that genes suspected of increasing the selective growth advantage of tumor cells be categorized as either "Mut-driver genes" or "Epi-driver genes." Mut-driver genes contain a sufficient number or type of driver gene mutations to unambiguously distinguish them from other genes. Epi-driver genes are expressed aberrantly in tumors but not frequently mutated; they are altered through changes in DNA methylation or chromatin modification that persist as the tumor cell divides.

A Ratiometric Method to Identify and Classify Mut-Driver Genes

If mutation frequency, corrected for mutation context, gene length, and other parameters, cannot reliably identify modestly mutated driver genes, what can? In our experience, the best way to identify Mut-driver genes is through their pattern of mutation rather than through their mutation frequency. The patterns of mutations in well-studied oncogenes and tumor suppressor genes are highly characteristic and nonrandom. Oncogenes are recurrently mutated at the same amino acid positions, whereas tumor suppressor genes are mutated through protein-truncating alterations throughout their length (Fig. 4 and table S2A).

On the basis of these mutation patterns rather than frequencies, we can determine which of the 18,306 mutated genes containing a total of 404,863 subtle mutations that have been recorded in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (*30*) are Mut-driver genes and whether they are likely to function as oncogenes or tumor suppressor genes. To be classified as an oncogene, we simply require that >20% of the recorded mutations in the gene are at recurrent positions and are missense (see legend to table S2A). To be classified as a tumor suppressor gene, we analogously require that >20% of the recorded mutations in the gene are inactivating. This "20/20 rule" is lenient in that all well-documented cancer genes far surpass these criteria (table S2A).

The following examples illustrate the value of the 20/20 rule. When *IDH1* mutations were first identified in brain tumors, their role in tumorigenesis was unknown (*2*, *31*). Initial functional studies suggested that *IDH1* was a tumor suppressor gene and that mutations inactivated this gene (*32*). However, nearly all of the mutations in *IDH1* were at the identical amino acid, codon 132 (Fig. 4). As assessed by the 20/20 rule, this distribution unambiguously indicated that *IDH1* was an oncogene rather than a tumor suppressor gene, and this conclusion was eventually supported by biochemical experiments (*33*, *34*). Another example is provided by mutations in *NOTCH1*. In this case, some functional studies suggested that *NOTCH1* was an oncogene, whereas others suggested it was a tumor suppressor gene (*35*, *36*). The situation could be clarified through the application of the 20/20 rule to *NOTCH1* mutations in cancers. In "liquid tumors" such as lymphomas and leukemias, the mutations were often recurrent and did not truncate the predicted protein (*37*). In squamous cell carcinomas, the mutations were not recurrent and were usually inactivating (*38*–*40*). Thus, the genetic data clearly indicated that *NOTCH1* functions differently in different tumor types. The idea that the same gene can function

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in completely opposite ways in different cell types is important for understanding cell signaling pathways.

How Many Mut-Driver Genes Exist?

Though all 20,000 protein-coding genes have been evaluated in the genome-wide sequencing studies of 3284 tumors, with a total of 294,881 mutations reported, only 125 Mut-driver genes, as defined by the 20/20 rule, have been discovered to date (table S2A). Of these, 71 are tumor suppressor genes and 54 are oncogenes. An important but relatively small fraction (29%) of these genes was discovered to be mutated through unbiased genome-wide sequencing; most of these genes had already been identified by previous, more directed investigations.

How many more Mut-driver genes are yet to be discovered? We believe that a plateau is being reached, because the same Mut-driver genes keep being "rediscovered" in different tumor types. For example, *MLL2* and *MLL3* mutations were originally discovered in medulloblastomas (*41*) and were subsequently discovered to be mutated in non-Hodgkin lymphomas, prostate cancers, breast cancers, and other tumor types (*42*–*45*). Similarly, *ARID1A* mutations were first discovered to be mutated in clear-cell ovarian cancers (*46*, *47*) and were subsequently shown to be mutated in tumors of several other organs, including those of the stomach and liver (*48*–*50*). In recent studies of several types of lung cancer (*4*, *51*, *52*), nearly all genes found to be mutated at significant frequencies had already been identified in tumors of other organs. In other words, the number of frequently altered Mut-driver genes (mountains) is nearing saturation. More mountains will undoubtedly be discovered, but these will likely be in uncommon tumor types that have not yet been studied in depth.

The newly discovered Mut-driver genes that have been detected through genome-wide sequencing have often proved illuminating. For example, nearly half of these genes encode proteins that directly regulate chromatin through modification of histones or DNA. Examples include the histones HIST1H3B and H3F3A, as well as the proteins DNMT1 and TET1, which covalently modify DNA, EZH2, SETD2, and KDM6A, which, in turn, methylate or demethylate histones (*53*–*57*). These discoveries have profound implications for understanding the mechanistic basis of the epigenetic changes that are rampant in tumors (*58*). The discovery of genetic alterations in genes encoding mRNA splicing factors, such as *SF3B1* and *U2AF1* (*59*–*61*), was similarly stunning, as mutations in these genes would be expected to lead to a plethora of nonspecific cellular stresses rather than to promote specific tumor types. Another example is provided by mutations in the cooperating proteins ATRX and DAXX (*62*). Tumors with mutations in these genes all have a specific type of telomere elongation process termed "ALT" (for "alternative lengthening of telomeres") (*63*). Though the ALT phenotype had been recognized for more than a decade, its genetic basis was mysterious before the discovery of mutations of these genes and their perfect correlation with the ALT phenotype (*64*). A final example is provided by *IDH1* and *IDH2*, whose mutations have stimulated the burgeoning field of tumor metabolism (*65*) and have had fascinating implications for epigenetics (*66*, *67*).

The Mut-driver genes listed in table S2A are affected by subtle mutations: base substitutions, intragenic insertions, or deletions. As noted above, Mut-driver genes can also be altered by less subtle changes, such as translocations, amplifications, and large-scale deletions. As with point mutations, it can be difficult to distinguish Mut-driver genes that are altered by these types of changes from genes that contain only passenger mutations. Genes that are not point-mutated, but are recurrently amplified (e.g., *MYC* family genes) or homozygously deleted (e.g., *MAP2K4*) and that meet other criteria (e.g., being the only gene in the amplicon or homozygously deleted region) are listed in table S2B. This adds 13 Mut-driver genes—10 oncogenes that are amplified and 3 tumor suppressor genes that are homozygously deleted—to the 125 driver genes that are affected by subtle mutations, for a total of 138 driver genes discovered to date (table S2).

Translocations provide similar challenges for driver classification. An important discovery related to this point is chromothripsis (*68*), a rare cataclysmic event involving one or a small number of chromosomes that results in a large number of chromosomal rearrangements. This complicates any inferences about causality, in the same way that mismatch repair deficiency compromises the interpretation of point mutations. However, for completeness, all fusion genes that have been identified in at least three independent tumors are listed in table S3. Virtually all of these genes were discovered through conventional approaches before the advent of genome-wide DNA sequencing studies, with some notable exceptions such as those described in (*6*) and (*69*). The great majority of these translocations are found in liquid tumors (leukemias and lymphomas) (table S3C) or mesenchymal tumors (table S3B) and were initially identified through karyotypic analyses. A relatively small number of recurrent fusions, the most important of which include *ERG* in prostate cancers (*70*) and *ALK* in lung cancers (*71*), have been described in more common tumors (table S3A).

Genes exist that predispose to cancer when inherited in mutant form in the germ line, but are not

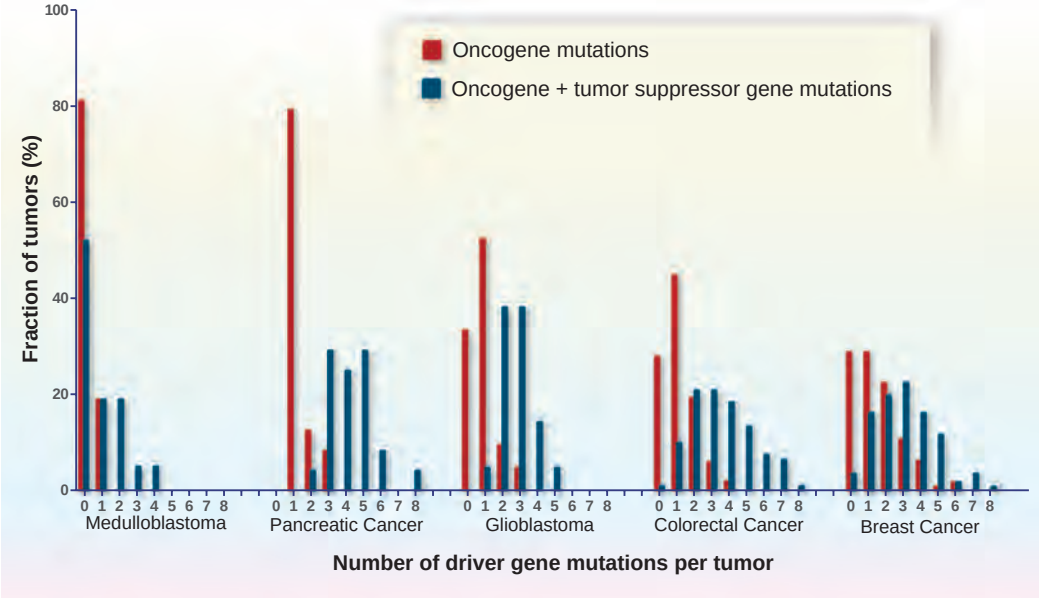

Fig. 5. Number and distribution of driver gene mutations in five tumor types. The total number of driver gene mutations [in oncogenes and tumor suppressor genes (TSGs)] is shown, as well as the number of oncogene mutations alone. The driver genes are listed in tables S2A and S2B. Translocations are not included in this figure, because few studies report translocations along with the other types of genetic alterations on a per-case basis. In the tumor types shown here, translocations affecting driver genes occur in less than 10% of samples. The published data on which this figure is based are provided in table S1E.

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somatically mutated in cancer to a substantial degree. These genes generally do not confer an increase in selective growth advantage when they are abnormal, but they stimulate tumorigenesis in indirect ways (such as by increasing genetic instability, as discussed later in this Review). For completeness, these genes and the hereditary syndromes for which they are responsible are listed in table S4.

Dark Matter

Classic epidemiologic studies have suggested that solid tumors ordinarily require five to eight "hits," now interpreted as alterations in driver genes, to develop (*72*). Is this number compatible with the molecular genetic data? In pediatric tumors such as medulloblastomas, the number of driver gene mutations is low (zero to two), as expected from the discussion above (Fig. 5). In common adult tumors—such as pancreatic, colorectal, breast, and brain cancers—the number of mutated driver genes is often three to six, but several tumors have only one or two driver gene mutations (Fig. 5). How can this be explained, given the widely accepted notion that tumor development and progression require multiple, sequential genetic alterations acquired over decades?

First, technical issues explain some of the "missing mutations." Genome-wide sequencing is far from perfect, at least with the technologies available today. Some regions of the genome are not well represented because their sequences are difficult to amplify, capture, or unambiguously map to the genome (*73*–*76*). Second, there is usually a wide distribution in the number of times that a specific nucleotide in a given gene is observed in the sequence data, so some regions will not be well represented by chance factors alone (*77*). Finally, primary tumors contain not only neoplastic cells, but also stromal cells that dilute the signal from the mutated base, further reducing the probability of finding a mutation (*78*).

What fraction of mutations are missed by these three technical issues? A recent study of pancreatic cancers is informative in this regard. Biankin *et al*. used immunohistochemical and genetic analyses to select a set of primary tumor samples enriched in neoplastic cells (*79*). They used massively parallel sequencing to analyze the exomes of these samples, then compared their mutational data with a set of pancreatic cancer cell lines and xenografts in which mutations had previously been identified, using conventional Sanger sequencing, and confirmed to be present in the primary tumors (*3*, *16*). Only 159 (63%) of the expected 251 driver gene mutations were identified in the primary tumors studied by next-generation sequencing alone, indicating a false-negative rate of 37%. Genome-wide studies in which the proportion of neoplastic cells within tumors is not as carefully evaluated as in (*79*) will have higher false-negative rates. Moreover, these technical problems are exacerbated in whole-genome studies compared with exomic analyses, because the sequence coverage of the former is often lower than that of the latter (generally 30-fold in whole-genome studies versus more than 100-fold in exomic studies).

Conceptual issues also limit the number of detectable drivers. Virtually all studies, either at the whole-genome or whole-exome level, have focused on the coding regions. The reason for this is practical; it is difficult enough to identify driver gene mutations when they qualitatively alter the sequence of the encoded protein. Trying to make sense of intergenic or intronic mutations is much more difficult. Based on analogous studies of the identifiable mutations in patients with monogenic diseases, more than 80% of mutations should be detectable through analysis of the coding regions (*80*). However, this still leaves some mutations as unidentifiable "dark matter," even in the germline genomes of heritable cases, which are usually easier to interpret than the somatic mutations in cancers. The first examples of light coming to such dark matter have recently been published: Recurrent mutations in the promoter of the *TERT* gene, encoding the catalytic subunit of telomerase, have been identified and shown to activate its transcription (*81*, *82*).

Mut-driver genes other than those listed in table S2 will undoubtedly be discovered as genome-wide sequencing continues. However, based on the trends noted above, most of the Mut-driver genes will likely be mountains in rare tumor types or small hills in common tumor types; thus, these genes are unlikely to account for the bulk of the presumptive dark matter. Other types of dark matter can be envisioned, however. Copy-number alterations are ubiquitous in cancers, at either the whole-chromosome or subchromosomal levels. These alterations could subtly change the expression of their driver genes. Recent studies have suggested that the loss of one copy of chromosomes containing several tumor suppressor genes, each plausibly connected to neoplasia but not altered by

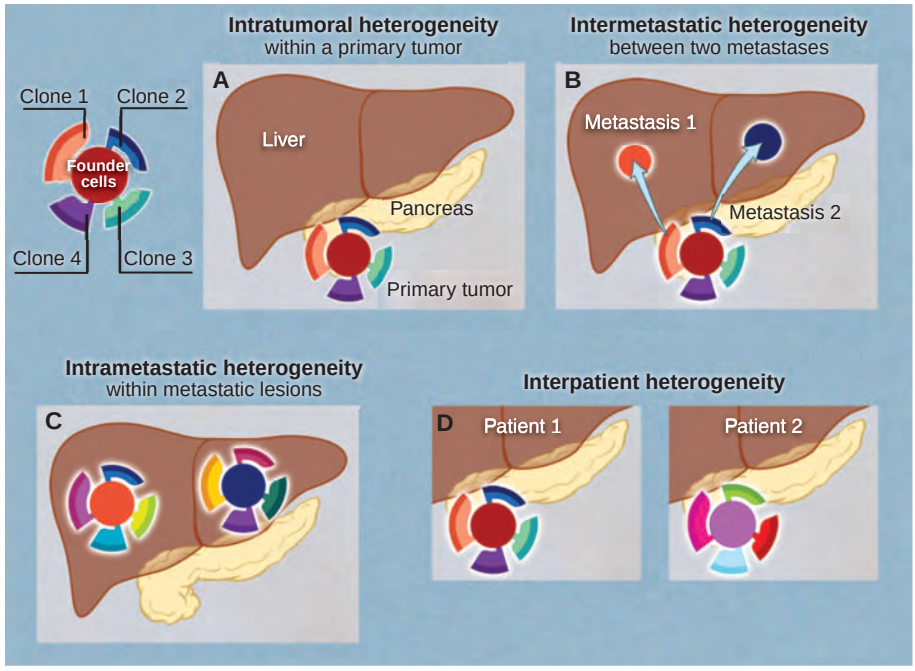

Fig. 6. Four types of genetic heterogeneity in tumors, illustrated by a primary tumor in the pancreas and its metastatic lesions in the liver. Mutations introduced during primary tumor cell growth result in clonal heterogeneity. At the top left, a typical tumor is represented by cells with a large fraction of the total mutations (founder cells) from which subclones are derived. The differently colored regions in the subclones represent stages of evolution within a subclone. (**A**) Intratumoral: heterogeneity among the cells of the primary tumor. (**B**) Intermetastatic: heterogeneity among different metastatic lesions in the same patient. In the case illustrated here, each metastasis was derived from a different subclone. (**C**) Intrametastatic: heterogeneity among the cells of each metastasis develops as the metastases grow. (**D**) Interpatient: heterogeneity among the tumors of different patients. The mutations in the founder cells of the tumors of these two patients are almost completely distinct (see text).

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mutation, may confer a selective growth advantage (*83*, *84*).

The most obvious source of dark matter is in Epi-driver genes. Human tumors contain large numbers of epigenetic changes affecting DNA or chromatin proteins. For example, a recent study of colorectal cancers showed that more than 10% of the protein-coding genes were differentially methylated when compared with normal colorectal epithelial cells (*85*). Some of these changes (i.e., those in Epi-driver genes) are likely to provide a selective growth advantage (*86*, *87*). For example, epigenetic silencing of *CDK2NA* and *MLH1* is much more common than mutational inactivation of either of these two well-recognized driver genes (*85*) However, there is a critical difference between a genetic and an epigenetic change in a gene. Unlike the sequence of a gene in a given individual, methylation is plastic, varying with cell type, developmental stage, and patient age (*21*). The methylation state of the normal precursor cells that initiate tumorigenesis is unknown; these cells, such as normal stem cells, may represent only a tiny fraction of the cells in a normal organ. This plasticity also means that methylation can change under microenvironmental cues, such as those associated with low nutrient concentrations or abnormal cell contacts. It is therefore difficult to know whether specific epigenetic changes observed in cancer cells reflect, rather than contribute to, the neoplastic state. Criteria for distinguishing epigenetic changes that exert a selective growth advantage from those that do not (passenger epigenetic changes) have not yet been formulated. Given that Epi-driver genes are likely to compose a major component of the dark matter, further research on this topic is essential (*58*).

Genetic Heterogeneity

The mutations depicted in Fig. 1 are clonal; that is, they are present in the majority of the neoplastic cells in the tumors. But additional, subclonal (i.e., heterogeneous within the tumor) mutations are important for understanding tumor evolution. Four types of genetic heterogeneity are relevant to tumorigenesis (Fig. 6):

1) Intratumoral: heterogeneity among the cells of one tumor. This type of heterogeneity has been recognized for decades. For example, it is rare to see a cytogenetic study of a solid tumor in which all of the tumor cells display the same karyotype (*88*). The same phenomenon has been noted for individual genes [e.g., (*89*)] and more recently has been observed throughout the genome (*16*, *90*–*96*). This kind of heterogeneity must exist: Every time a normal (or tumor) cell divides, it acquires a few mutations, and the number of mutations that distinguish any two cells simply marks the time from their last common ancestor (their founder cell). Cells at the opposite ends of large tumors will be spatially distinct and, in general, will display more differences than neighboring cells (*16*). This phenomenon is analogous to speciation, wherein organisms on different islands are more likely to diverge from one another than are organisms on the same island.

In studies that have evaluated intratumoral heterogeneity by genome-wide sequencing, the majority of somatic mutations are present in all tumor cells. These mutations form the trunk of the somatic evolutionary tree. What is the importance of the mutations in the branches (i.e., those that are not shared by all tumor cells)? From a medical perspective, these mutations are often meaningless because the primary tumors are surgically removed. How much heterogeneity existed in the various branches before surgery is not important. However, this heterogeneity provides the seeds for intermetastastic heterogeneity, which is of great clinical importance.

2) Intermetastatic: heterogeneity among different metastatic lesions of the same patient. The vast majority of cancer patients die because their tumors were not removed before metastasis to surgically inaccessible sites, such as the liver, brain, lung, or bone. Patients who relapse with a single metastatic lesion can often still be cured by surgery or radiotherapy, but single metastases are the exception rather than the rule. A typical patient on a clinical trial has a dozen or more metastatic lesions large enough to be visualized by imaging, and many more that are smaller. If each of the metastatic lesions in a single patient was founded by a cell with a very different genetic constitution, then chemotherapeutic cures would be nearly impossible to achieve: Eradicating a subset of the metastatic lesions in a patient will not be adequate for long-term survival.

How much heterogeneity is there among different metastatic lesions? In short, a lot. It is not uncommon for one metastatic lesion to have 20 clonal genetic alterations not shared by other metastases in the same patient (*16*, *97*). Because they are clonal, these mutations occurred in the founder cell of the metastasis; that is, the cell that escaped from the primary tumor and multiplied to form the metastasis. The founder cell for each metastasis is present in different, geographically distinct areas of the primary tumors, as expected (*16*).

This potentially disastrous situation is tempered by the fact that the heterogeneity appears largely confined to passenger gene mutations. In most of the studies documenting heterogeneity in malignancies, the Mut-driver genes are present in the trunks of the trees, though exceptions have been noted (*95*). These findings are consistent with the idea, discussed above, that the genetic alterations required for metastasis were present (i.e., selected for) before metastasis actually occurred. The data are also consistent with the observation that in patients responsive to targeted agents, the response is often seen in all metastatic lesions rather than just a small subset (*98*).

3) Intrametastatic: heterogeneity among the cells of an individual metastasis. Each metastasis is established by a single cell (or small group of cells) with a set of founder mutations. As it grows, the metastasis acquires new mutations with each cell division. Though the founder mutations may make the lesion susceptible to antitumor agents, the new mutations provide the seeds for drug resistance. Unlike primary tumors, the metastatic lesions generally cannot be removed by surgery and must be treated with systemic therapies. Patients with complete responses to targeted therapies invariably relapse. Most of the initial lesions generally recur, and the time frame at which they recur is notably similar. This time course can be explained by the presence of resistance mutations that existed within each metastasis before the onset of the targeted therapy (*99*–*102*). Calculations show that any metastatic lesion of a size visible on medical imaging has thousands of cells (among the billions present) that are already resistant to virtually any drug that can be imagined (*99*, *101*, *102*). Thus, recurrence is simply a matter of time, entirely predictable on the basis of known mutation frequencies and tumor cell growth rates. This "fait accompli" can be circumvented, in principle, by treatment with multiple agents, as it is unlikely that a single tumor cell will be resistant to multiple drugs that act on different targets.

4) Interpatient: heterogeneity among the tumors of different patients. This type of heterogeneity has been observed by every oncologist; no two cancer patients have identical clinical courses, with or without therapy. Some of these differences could be related to host factors, such as germline variants that determine drug half-life or vascular permeability to drugs or cells, and some could be related to nongenetic factors (*103*). However, much of this interpatient heterogeneity is probably related to somatic mutations within tumors. Though several dozen somatic mutations may be present in the breast cancers from two patients, only a small number are in the same genes, and in the vast majority of cases, these are the Mut-driver genes (*1*, *104*, *105*). Even in these driver genes, the actual mutations are often different. Mutations altering different domains of a protein would certainly not be expected to have identical effects on cellular properties, as experimentally confirmed (*106*). Though it may seem that different mutations in adjacent codons would have identical effects, detailed studies of large numbers of patients have shown that this need not be the case. For example, a $Gly^{12} \rightarrow Asp^{12}$ (G12D) mutation of *KRAS* does not have the same clinical implications as a G13D mutation of the same gene (*107*). Interpatient heterogeneity has always been one of the major obstacles

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to designing uniformly effective treatments for cancer. Efforts to individualize treatments based on knowledge of the genomes of cancer patients are largely based on an appreciation of this heterogeneity.

Signaling Pathways in Tumors

The immense complexity of cancer genomes that could be inferred from the data described above is somewhat misleading. After all, even advanced tumors are not completely out of control, as evidenced by the dramatic responses to agents that target mutant *BRAF* in melanomas (*108*) or mutant *ALK* in lung cancers (*109*). Albeit transient, these responses mean that interference with even a single mutant gene product is sufficient to stop cancer in its tracks, at least transiently. How can the genomic complexity of cancer be reconciled with these clinical observations?

Two concepts bear on this point. The first, mentioned above, is that >99.9% of the alterations in tumors (including point mutations, copy-number alterations, translocations, and epigenetic changes distributed throughout the genome, not just in the coding regions) are immaterial to neoplasia. They are simply passenger changes that mark the time that has elapsed between successive clonal expansions. Normal cells also undergo genetic alterations as they divide, both at the nucleotide and chromosomal levels. However, normal cells are programmed to undergo cell death in response to such alterations, perhaps as a protective mechanism against cancer. In contrast, cancer cells have evolved to tolerate genome complexity by acquiring mutations in genes such as *TP53* (*110*). Thus, genomic complexity is, in part, the result of cancer, rather than the cause.

To appreciate the second concept, one must take the 30,000-foot view. A jungle might look chaotic at ground level, but the aerial view shows a clear order, with all the animals gathering at the streams at certain points in the day, and all the streams converging at a river. There is order in cancer, too. Mutations in all of the 138 driver genes listed in table S2 do one thing: cause a selective growth advantage, either directly or indirectly. Moreover, there appears to be only a limited number of cellular signaling pathways through which a growth advantage can be incurred (Fig. 7 and table S5).

All of the known driver genes can be classified into one or more of 12 pathways (Fig. 7). The discovery of the molecular components of these pathways is one of the greatest achievements of biomedical research, a tribute to investigators working in fields that encompass biochemistry, cell biology, and development, as well as cancer. These pathways can themselves be further organized into three core cellular processes:

1) Cell fate: Numerous studies have demonstrated the opposing relationship between cell division and differentiation, the arbiters of cell fate. Dividing cells that are responsible for populating normal tissues (stem cells) do not differentiate, and vice versa. Regenerative medicine is based on this distinction, predicated on ways to get differentiated cells to dedifferentiate into stem cells, then forcing the stem cells to differentiate into useful cell types for transplantation back into the patient. Many of the genetic alterations in cancer abrogate the precise balance between differentiation and division, favoring the latter. This causes a selective growth advantage, because differentiating cells eventually die or become quiescent. Pathways that function through this process include APC, HH, and NOTCH, all of which are well known to control cell fate in organisms ranging from worms to mammals (*111*). Genes encoding chromatin-modifying enzymes can also be included in this category. In normal development, the heritable switch from division to differentiation is not determined by mutation, as it is in cancer, but rather by epigenetic alterations affecting DNA and chromatin proteins. What better way to subvert this normal mechanism for controlling tissue architecture than to debilitate the epigenetic modifying apparatus itself?

2) Cell survival: Though cancer cells divide abnormally because of cell-autonomous alterations, such as those controlling cell fate, their surrounding stromal cells are perfectly normal and do not keep pace. The most obvious ramification of this asymmetry is the abnormal vasculature of tumors. As opposed to the well-ordered network of arteries, veins, and lymphatics that control nutrient concentrations in normal tissues, the vascular system in cancers is tortuous and lacks uniformity of structure (*112*, *113*). Normal cells are always within 100 μm of a capillary, but this is not true for cancer cells (*114*). As a result, a cancer cell acquiring a mutation that allows it to proliferate under limiting nutrient concentrations will have a selective growth advantage, thriving in environments in which its sister cells cannot. Mutations of this sort occur, for example, in the *EGFR*, *HER2*, *FGFR2*, *PDGFR*, *TGFβR2*, *MET*, *KIT*, *RAS*, *RAF*, *PIK3CA*, and *PTEN* genes (table S2A). Some of these genes encode receptors for the growth factors themselves, whereas others relay the signal from the growth factor to the interior of the cell, stimulating growth when activated (*115*, *116*). For instance, mutations in *KRAS* or *BRAF* genes confer on cancer cells the ability to grow in glucose concentrations that are lower than those required for the growth of normal cells or of cancer cells that do not have mutations in these genes (*117*, *118*). Progression through the cell cycle (and its antithesis, apoptosis) can be directly controlled by intracellular metabolites, and driver genes that directly regulate the cell cycle or apoptosis, such as *CDKN2A*, *MYC*, and *BCL2*, are often mutated in cancers. Another gene whose mutations enhance cell survival is *VHL*, the product of which stimulates angiogenesis through the secretion of vascular endothelial growth factor. What better way to provision growth factors to a rogue tumor than to lure the unsuspecting vasculature to its hideout?

3) Genome maintenance: As a result of the exotic microenvironments in which they reside, cancer cells are exposed to a variety of toxic substances, such as reactive oxygen species. Even without microenvironmental poisons, cells make mistakes while replicating their DNA or during division (*119*, *120*), and checkpoints exist to either slow down such cells or make them commit suicide (apoptosis) under such circumstances (*110*, *121*, *122*). Although it is good for the organism to remove these damaged cells, tumor cells that can survive the damage will, by definition, have a selective growth advantage. Therefore, it is not surprising that genes whose mutations abrogate these checkpoints, such as *TP53* and *ATM*, are mutated in cancers

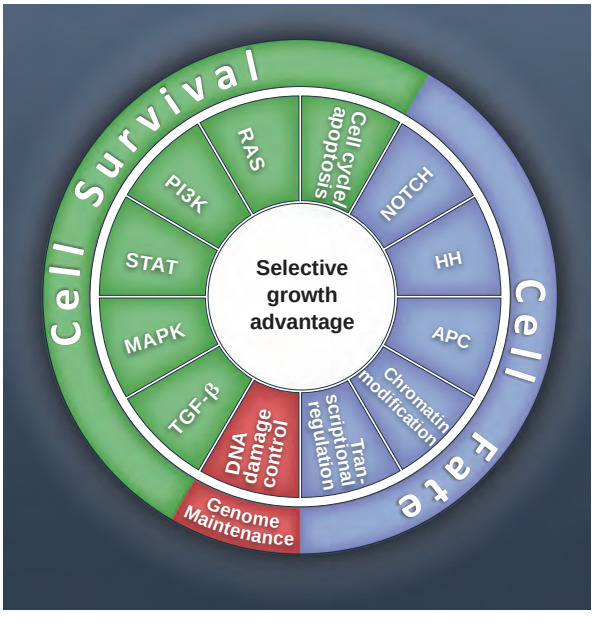

Fig. 7. Cancer cell signaling pathways and the cellular processes they regulate. All of the driver genes listed in table S2 can be classified into one or more of 12 pathways (middle ring) that confer a selective growth advantage (inner circle; see main text). These pathways can themselves be further organized into three core cellular processes (outer ring). The publications on which this figure is based are provided in table S5.

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(*123*). Defects in these genes can also indirectly confer a selective growth advantage by allowing cells that have a gross chromosomal change favoring growth, such as a translocation or an extra chromosome, to survive and divide. Analogously, genes that control point mutation rates, such as *MLH1* or *MSH2*, are mutated in cancers (table S2A) or in the germ line of patients predisposed to cancers (table S4) because they accelerate the acquisition of mutations that function through processes that regulate cell fate or survival. What better way to promote cancer than by increasing the rate of occurrence of the mutations that drive the process?

Because the protein products of genes regulating cell fate, cell survival, and genome maintenance often interact with one another, the pathways within them overlap; they are not as discrete as might be inferred from the description above. However, grouping genes into pathways makes perfect sense from a genetics standpoint. Given that cancer is a genetic disease, the principles of genetics should apply to its pathogenesis. When performing a conventional mutagenesis screen in bacteria, yeast, fruit flies, or worms, one expects to discover mutations in several different genes that confer similar phenotypes. The products of these genes often interact with one another and define a biochemical or developmental pathway. Therefore, it should not be surprising that several different genes can result in the same selective growth advantage for cancer cells and that the products of these genes interact. The analogy between cancer pathways and biochemical or developmental pathways in other organisms goes even deeper: The vast majority of our knowledge of the function of driver genes has been derived from the study of the pathways through which their homologs work in nonhuman organisms. Though the functions are not identical to those in human cells, they are highly related and have provided the starting point for analogous studies in human cells.

Recognition of these pathways also has important ramifications for our ability to understand interpatient heterogeneity. One lung cancer might have an activating mutation in a receptor for a stimulatory growth factor, making it able to grow in low concentrations of epidermal growth factor (EGF). A second lung cancer might have an activating mutation in *KRAS*, whose protein product normally transmits the signal from the epidermal growth factor receptor (EGFR) to other cell signaling molecules. A third lung cancer might have an inactivating mutation in *NF1*, a regulatory protein that normally inactivates the KRAS protein. Finally, a fourth lung cancer might have a mutation in *BRAF*, which transmits the signal from KRAS to downstream kinases (Fig. 8). One would predict that mutations in the various components of a single pathway would be mutually exclusive—that is, not occurring in the same tumor—and this has been experimentally confirmed (*124*, *125*). Apart from being intellectually satisfying, knowledge of these pathways has implications for cancer therapy, as discussed in the next section.

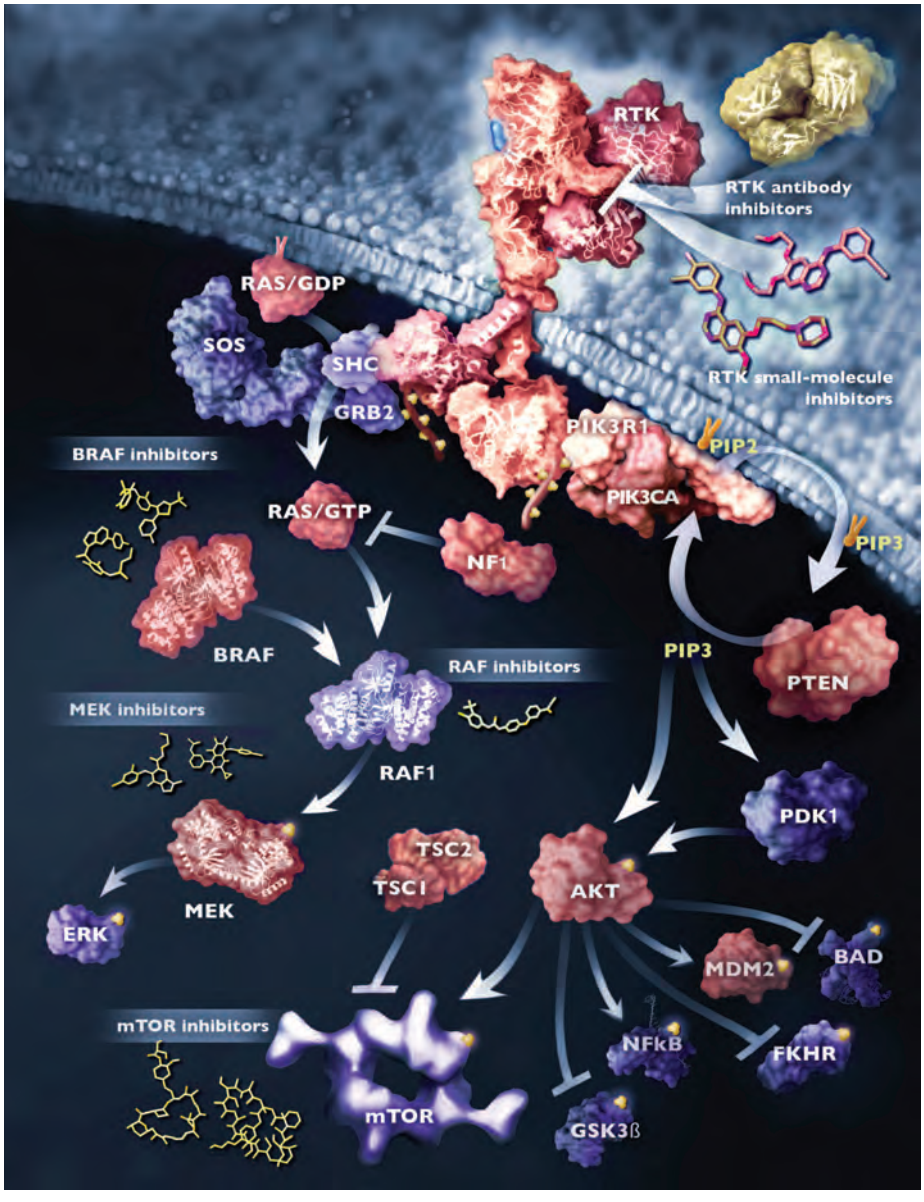

Fig. 8. Signal transduction pathways affected by mutations in human cancer. Two representative pathways from Fig. 7 (RAS and PI3K) are illustrated. The signal transducers are color coded: red indicates protein components encoded by the driver genes listed in table S2; yellow balls denote sites of phosphorylation. Examples of therapeutic agents that target some of the signal transducers are shown. RTK, receptor tyrosine kinase; GDP, guanosine diphosphate; MEK, MAPK kinase; ERK, extracellular signal–regulated kinase; NFkB, nuclear factor κB; mTOR, mammalian target of rapamycin.

CREDIT: FIG. 8, A. DIXON

A Perspective on Genome-Based Medicine in Oncology

Opportunities

Though cancer genome sequencing is a relatively new endeavor, it has already had an impact on the clinical care of cancer patients. The recognition that certain tumors contain activating mutations in driver genes encoding protein kinases has led to the development of small-molecule inhibitor drugs targeting those kinases.

Representative examples of this type of genome-based medicine include the use of EGFR kinase inhibitors to treat cancers with *EGFR* gene mutations (*126*), the aforementioned anaplastic lymphoma kinase (ALK) inhibitors to treat cancers with *ALK* gene translocations (*109*), and specific inhibitors of mutant BRAF

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to treat cancers with *BRAF* mutations (*108*). Before instituting treatment with such agents, it is imperative to determine whether the cancer harbors the mutations that the drug targets. Only a small fraction of lung cancer patients have *EGFR* gene mutations or *ALK* gene translocations, and only these patients will respond to the drugs. Treating lung cancer patients without these particular genetic alterations would be detrimental, as such patients would develop the toxic side effects of the drugs while their tumors progressed.

A second type of genome-based medicine focuses on the side effects and metabolism of the therapeutic agents, rather than the genetic alterations they target. At present, the dose of cancer drugs given to patients is based on the patients' size (body weight or surface area). But the therapeutic ratio of cancer drugs (ratio of the concentration that causes side effects to the concentration required to kill tumor cells) is generally low, particularly for conventional (nontargeted) therapeutic agents. Small changes in circulating concentrations of these drugs can make the difference between substantial tumor regression and intolerable side effects. Interrogation of the germline status of the genes encoding drug-metabolizing enzymes could substantially improve the outcomes of treatment by informing drug dosing (*127*). Optimally, this genome interrogation would be accompanied by pharmacokinetic measurements of drug concentrations in each patient. The additional cost of such analyses would be small compared with the exorbitant costs of new cancer therapies—for recently approved drugs, the cost is estimated to be $200,000 to $300,000 per quality life year produced (*128*).

Challenges

One challenge of genome-based medicine in oncology is already apparent from the opportunities described above: All of the clinically approved drugs that target the products of genetically altered genes are directed against kinases. One reason for this is that kinases are relatively easy to target with small molecules and have been extensively studied at the biochemical, structural, and physiologic levels (*129*). But another reason has far deeper ramifications. The vast majority of drugs on the market today, for cancer or other diseases, inhibit the actions of their protein targets. This inhibition occurs because the drugs interfere with the protein's enzymatic activity (such as the phosphorylation catalyzed by kinases) or with the binding of the protein to a small ligand (such as with G protein–coupled receptors). Only 31 of the oncogenes listed in tables S2 and S3 have enzymatic activities that are targetable in this manner. Many others participate in protein complexes, involving large interfaces and numerous weak interactions. Inhibiting the function of such proteins with small drugs is notoriously difficult because small compounds can only inhibit one of these interactions (*130*, *131*).

Though one can at least imagine the development of drugs that inhibit nonenzymatic protein functions, the second challenge evident from table S2 poses even greater difficulties: A large fraction of the Mut-driver genes encode tumor suppressors. Drugs generally interfere with protein function; they cannot, in general, replace the function of defective genes such as those resulting from mutations in tumor suppressor genes. Unfortunately, tumor suppressor gene–inactivating mutations predominate over oncogene-activating mutations in the most common solid tumors: Few individual tumors contain more than one oncogene mutation (Fig. 5).

The relatively small number of oncogene mutations in tumors is important in light of the intrametastatic heterogeneity described earlier. To circumvent the inevitable development of resistance to targeted therapies, it will likely be necessary to treat patients with two or more drugs. The probability that a single cancer cell within a large metastatic lesion will be resistant to two agents that target two independent pathways is exponentially less than the probability that the cell will be resistant to a single agent. However, if the cancer cell does not contain more than one targetable genetic alteration (i.e., an oncogene mutation), then this combination strategy is not feasible.

Given the paucity of oncogene alterations in common solid tumors and these principles, can targeted therapeutic approaches ever be expected to induce long-term remissions, even cures, rather than the short-term remissions now being achieved? The saviors are pathways; every tumor suppressor gene inactivation is expected to result in the activation of some growth-promoting signal downstream of the pathway. An example is provided by *PTEN* mutations: Inactivation of the tumor suppressor gene *PTEN* results in activation of the AKT kinase (Fig. 8). Similarly, inactivation of the tumor suppressor gene *CDKN2A* results in activation of kinases, such as cyclin-dependent kinase 4, that promote cell cycle traverse (*132*). Furthermore, inactivation of tumor suppressor gene *APC* results in constitutive activity of oncogenes such as *CTNNB1* and *CMYC* (*133*–*135*).

We believe that greater knowledge of these pathways and the ways in which they function is the most pressing need in basic cancer research. Successful research on this topic should allow the development of agents that target, albeit indirectly, defective tumor suppressor genes. Indeed, there are already examples of such indirect targeting. Inactivating mutations of the tumor suppressor genes *BRCA1* or *BRCA2* lead to activation of downstream pathways required to repair DNA damage in the absence of BRCA function. Thus, cancer cells with defects in *BRCA1* or *BRCA2* are more susceptible to DNA damaging agents or to drugs that inhibit enzymes that facilitate the repair of DNA damage such as PARP [poly(adenosine diphosphate–ribose) polymerase] (*136*). PARP inhibitors have shown

Box 2. Highlights

1. Most human cancers are caused by two to eight sequential alterations that develop over the course of 20 to 30 years.

2. Each of these alterations directly or indirectly increases the ratio of cell birth to cell death; that is, each alteration causes a selective growth advantage to the cell in which it resides.

3. The evidence to date suggests that there are ~140 genes whose intragenic mutations contribute to cancer (so-called Mut-driver genes). There are probably other genes (Epi-driver genes) that are altered by epigenetic mechanisms and cause a selective growth advantage, but the definitive identification of these genes has been challenging.

4. The known driver genes function through a dozen signaling pathways that regulate three core cellular processes: cell fate determination, cell survival, and genome maintenance.

5. Every individual tumor, even of the same histopathologic subtype as another tumor, is distinct with respect to its genetic alterations, but the pathways affected in different tumors are similar.

6. Genetic heterogeneity among the cells of an individual tumor always exists and can impact the response to therapeutics.

7. In the future, the most appropriate management plan for a patient with cancer will be informed by an assessment of the components of the patient's germline genome and the genome of his or her tumor.

8. The information from cancer genome studies can also be exploited to improve methods for prevention and early detection of cancer, which will be essential to reduce cancer morbidity and mortality.

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encouraging results in clinical trials when used in patients whose tumors have inactivating mutations of BRCA genes (*137*).

Further progress in this area will require more detailed information about the signaling pathways through which cancer genes function in human cancer cells, as well as in model organisms. One of the lessons of molecular biology over the past two decades is that pathway functions are different, depending on the organism, cell type, and precise genetic alterations in that cell (*138*). A pertinent example of this principle is provided by results of treatment with drugs inhibiting mutant BRAF kinase activity. In the majority of patients with melanomas harboring (V600E; V, Val; E, Glu) mutations in the *BRAF* gene, these drugs induce dramatic (though transient) remissions (*108*). But the same drugs have no therapeutic effect in colorectal cancer patients harboring the identical *BRAF* mutations (*139*). This observation has been attributed to the expression of EGFR, which occurs in some colorectal cancers but not in melanoma and is thought to circumvent the growth-inhibitory effects of the BRAF inhibitors. With this example in mind, no one should be surprised that a new drug that works well in an engineered tumor in mice fails in human trials; the organism is different, the cell type is usually different, and the precise genetic constitutions are always different. The converse of this statement—that a drug that fails in animal trials will not necessarily fail in human trials—has important practical consequences. In our view, if the biochemical and conceptual bases for a drug's actions are solid and the drug is shown to be safe in animals, then a human trial may be warranted, even if it does not shrink tumors in mice.

Genome-Based Medicines of the Future

Cancer genomes can also be exploited for the development of more effective immunotherapies. As noted above, typical solid tumors contain 30 to 70 mutations that alter the amino acid sequences of the proteins encoded by the affected genes. Each of these alterations is foreign to the immune system, as none have been encountered during embryonic or postnatal life. Therefore, these alterations, in principle, provide a "holy grail" for tumor immunology: truly tumor-specific antigens. These antigens could be incorporated into any of the numerous platforms that already exist for the immunotherapy of cancer. These include administration of vaccines containing the mutant peptide, viruses encoding the mutant peptides on their surfaces, dendritic cells presenting the mutated peptide, and antibodies or T cells with reactivity directed against the mutant peptides (*140*).

To realize these sorts of therapeutics, several conditions must be met. First, the mutant protein must be expressed. As cancer cells generally express about half of the proteins that are encoded by the human genome (*141*), this condition is not limiting. Second, as most proteins affected by mutations are intracellular, these mutations will not be visible to the immune system unless the mutant residue is presented in the context of a human leukocyte antigen (HLA) protein. Based on in silico analyses of binding affinities, it has been estimated that a typical breast or colorectal cancer contains 7 to 10 mutant proteins that can bind to an individual patient's HLA type (*142*). These theoretical predictions have recently gained experimental support. Studies of mouse tumors have identified mutant genes and shown that the corresponding peptides can induce antitumor immunity when administered as vaccines (*143*). Moreover, clinical trials of brain cancer patients immunized against a mutant peptide have yielded encouraging results (*144*).

As with all cancer therapies that are attractive in concept, obstacles abound in practice. If a tumor expresses a mutant protein that is recognizable as foreign, why has the host immune system not eradicated that tumor already? Indeed, immunoediting in cancers has been shown to exist, resulting in the down-regulation or absence of mutant epitopes that should have, and perhaps did, elicit an immune response during tumor development (*145*, *146*). Additionally, tumors can lose immunogenicity through a variety of genetic alterations, thereby precluding the presentation of epitopes that would otherwise be recognized as foreign (*147*). Though these theoretical limitations are disheartening, recent studies on immune regulation in humans portend cautious optimism (*148*, *149*).

Other Ways to Reduce Morbidity and Mortality Through Knowledge of Cancer Genomics

When we think about eradicating cancer, we generally think about curing advanced cases—those that cannot be cured by surgery alone because they have already metastasized. This is a curious way of thinking about this disease. When we think of cardiovascular or infectious diseases, we first consider ways to prevent them rather than drugs to cure their most advanced forms. Today, we are in no better position to cure polio or massive myocardial infarctions than we were a thousand years ago. But we can prevent these diseases entirely (vaccines), reduce incidence (dietary changes, statins), or mitigate severity (stents, thrombolytic agents) and thereby make a major impact on morbidity and mortality.

This focus on curing advanced cancers might have been reasonable 50 years ago, when the molecular pathogenesis of cancers was mysterious and when chemotherapeutic agents against advanced cancers were showing promise. But this mindset is no longer acceptable. We now know precisely what causes cancer: a sequential series of alterations in well-defined genes that alter the function of a limited number of pathways. Moreover, we know that this process takes decades to develop and that the incurable stage, metastasis, occurs only a few years before death. In other words, of the one million people that will die from cancer this year, the vast majority will die only because their cancers were not detected in the first 90% of the cancers' lifetimes, when they were amenable to the surgeons' scalpel.

This new knowledge of cancer (Box 2) has reinvigorated the search for cures for advanced cancers, but has not yet permeated other fields of applied cancer research. A common and limited set of driver genes and pathways is responsible for most common forms of cancer (table S2); these genes and pathways offer distinct potential for early diagnosis. The genes themselves, the proteins encoded by these genes, and the end products of their pathways are, in principle, detectable in many ways, including analyses of relevant body fluids, such as urine for genitourinary cancers, sputum for lung cancers, and stool for gastrointestinal cancers (*150*). Equally exciting are the possibilities afforded by molecular imaging, which not only indicate the presence of a cancer but also reveal its precise location and extent. Additionally, research into the relationship between particular environmental influences (diet and lifestyle) and the genetic alterations in cancer is sparse, despite its potential for preventative measures.

The reasons that society invests so much more in research on cures for advanced cancers than on prevention or early detection are complex. Economic issues play a part: New drugs are far more lucrative for industry than new tests, and large individual costs for treating patients with advanced disease have become acceptable, even in developing countries (*151*). From a technical standpoint, the development of new and improved methods for early detection and prevention will not be easy, but there is no reason to assume that it will be more difficult than the development of new therapies aimed at treating widely metastatic disease.

Our point is not that strenuous efforts to develop new therapies for advanced cancer patients should be abandoned. These will always be required, no matter our arsenal of early detection or preventative measures. Instead, we are suggesting that "plan A" should be prevention and early detection, and "plan B" (therapy for advanced cancers) should be necessary only when plan A fails. To make plan A viable, government and philanthropic organizations must dedicate a much greater fraction of their resources to this cause, with long-term considerations in mind. We believe that cancer deaths can be reduced by more than 75% in the coming decades (*152*), but that this reduction will only come about if greater efforts are made toward early detection and prevention.

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Supplementary Materials

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CANCERGENOMICS
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REVIEW

Cancer Genome Landscapes

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Over the past decade, comprehensive sequencing efforts have revealed the genomic landscapes of common forms of human cancer. For most cancer types, this landscape consists of a small number of "mountains" (genes altered in a high percentage of tumors) and a much larger number of "hills" (genes altered infrequently). To date, these studies have revealed ~140 genes that, when altered by intragenic mutations, can promote or "drive" tumorigenesis. A typical tumor contains two to eight of these "driver gene" mutations; the remaining mutations are passengers that confer no selective growth advantage. Driver genes can be classified into 12 signaling pathways that regulate three core cellular processes: cell fate, cell survival, and genome maintenance. A better understanding of these pathways is one of the most pressing needs in basic cancer research. Even now, however, our knowledge of cancer genomes is sufficient to guide the development of more effective approaches for reducing cancer morbidity and mortality.

Ten years ago, the idea that all of the genes altered in cancer could be identified at base-pair resolution would have seemed like science fiction. Today, such genome-wide analysis, through sequencing of the exome (see Box 1, Glossary, for definitions of terms used in this Review) or of the whole genome, is routine.

The prototypical exomic studies of cancer evaluated ~20 tumors at a cost of >$100,000 per case (*1*–*3*). Today, the cost of this sequencing has been reduced 100-fold, and studies reporting the sequencing of more than 100 tumors of a given type are the norm (table S1A). Although vast amounts of data can now be readily obtained, deciphering this information in meaningful terms is still challenging. Here, we review what has been learned about cancer genomes from these sequencing studies—and, more importantly, what this information has taught us about cancer biology and future cancer management strategies.

How Many Genes Are Subtly Mutated in a Typical Human Cancer?

In common solid tumors such as those derived from the colon, breast, brain, or pancreas, an average of 33 to 66 genes display subtle somatic mutations that would be expected to alter their protein products (Fig. 1A). About 95% of these mutations are single-base substitutions (such as C>G), whereas the remainder are deletions or insertions of one or a few bases (such as CTT>CT) (table S1B). Of the base substitutions, 90.7% result in missense changes, 7.6% result in nonsense changes, and 1.7% result in alterations of splice sites or untranslated regions immediately adjacent to the start and stop codons (table S1B).

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Certain tumor types display many more or many fewer mutations than average (Fig. 1B). Notable among these outliers are melanomas and lung tumors, which contain ~200 nonsynonymous mutations per tumor (table S1C). These larger numbers reflect the involvement of potent mutagens (ultraviolet light and cigarette smoke, respectively) in the pathogenesis of these tumor types. Accordingly, lung cancers from smokers have 10 times as many somatic mutations as those from nonsmokers (*4*). Tumors with defects in DNA repair form another group of outliers (*5*). For example, tumors with mismatch repair defects can harbor thousands of mutations (Fig. 1B), even more than lung tumors or melanomas. Recent studies have shown that high numbers of mutations are also found in tumors with genetic alterations of the proofreading domain of DNA polymerases POLE or POLD1 (*6*, *7*). At the other end of the spectrum, pediatric tumors and leukemias harbor far fewer point mutations: on average, 9.6 per tumor (table S1C). The basis for this observation is considered below.

Mutation Timing

When do these mutations occur? Tumors evolve from benign to malignant lesions by acquiring a series of mutations over time, a process that has been particularly well studied in colorectal tumors (*8*, *9*). The first, or "gatekeeping," mutation provides a selective growth advantage to a normal epithelial cell, allowing it to outgrow the cells that surround it and become a microscopic clone (Fig. 2). Gatekeeping mutations in the colon most often occur in the *APC* gene (*10*). The small adenoma that results from this mutation grows slowly, but a second mutation in another gene, such as *KRAS*, unleashes a second round of clonal growth that allows an expansion of cell number (*9*). The cells with only the *APC* mutation may persist, but their cell numbers are small compared with the cells that have mutations in both genes. This process of mutation followed by clonal expansion continues, with mutations in genes such as *PIK3CA*, *SMAD4*, and *TP53*, eventually generating a malignant tumor that can invade through the underlying basement membrane and metastasize to lymph nodes and distant organs such as the liver (*11*). The mutations that confer a selective growth advantage to the tumor cell are called "driver" mutations. It has been estimated (*12*) that each driver mutation provides only a small selective growth advantage to the cell, on the order of a 0.4% increase in the difference between cell birth and cell death. Over many years, however, this slight increase, compounded once or twice per week, can result in a large mass, containing billions of cells.

The number of mutations in certain tumors of self-renewing tissues is directly correlated with age (*13*). When evaluated through linear regression, this correlation implies that more than half of the somatic mutations identified in these tumors occur during the preneoplastic phase; that is, during the growth of normal cells that continuously replenish gastrointestinal and genitourinary epithelium and other tissues. All of these pre-neoplastic mutations are "passenger" mutations that have no effect on the neoplastic process. This result explains why a colorectal tumor in a 90-year-old patient has nearly twice as many mutations as a morphologically identical colorectal tumor in a 45-year-old patient. This finding also partly explains why advanced brain tumors (glioblastomas) and pancreatic cancers (pancreatic ductal adenocarcinomas) have fewer mutations than colorectal tumors; glial cells of the brain and epithelial cells of the pancreatic ducts do not replicate, unlike the epithelial cells lining the crypts of the colon. Therefore, the gatekeeping mutation in a pancreatic or brain cancer is predicted to occur in a precursor cell that contains many fewer mutations than are present in a colorectal precursor cell. This line of reasoning also helps to explain why pediatric cancers have fewer mutations than adult tumors. Pediatric cancers often occur in non–self-renewing tissues, and those that arise in renewing tissues (such as leukemias) originate from precursor cells that have not renewed themselves as often as in adults. In addition, pediatric tumors, as well as adult leukemias and lymphomas, may require fewer rounds of clonal expansion than adult solid tumors (*8*, *14*). Genome sequencing studies of leukemia patients support the idea that mutations occur as random events in normal precursor cells before these cells acquire an initiating mutation (*15*).

When during tumorigenesis do the remaining somatic mutations occur? Because mutations in tumors occur at predictable and calculable rates (see below), the number of somatic mutations in tumors provides a clock, much like the clock used in evolutionary biology to determine species

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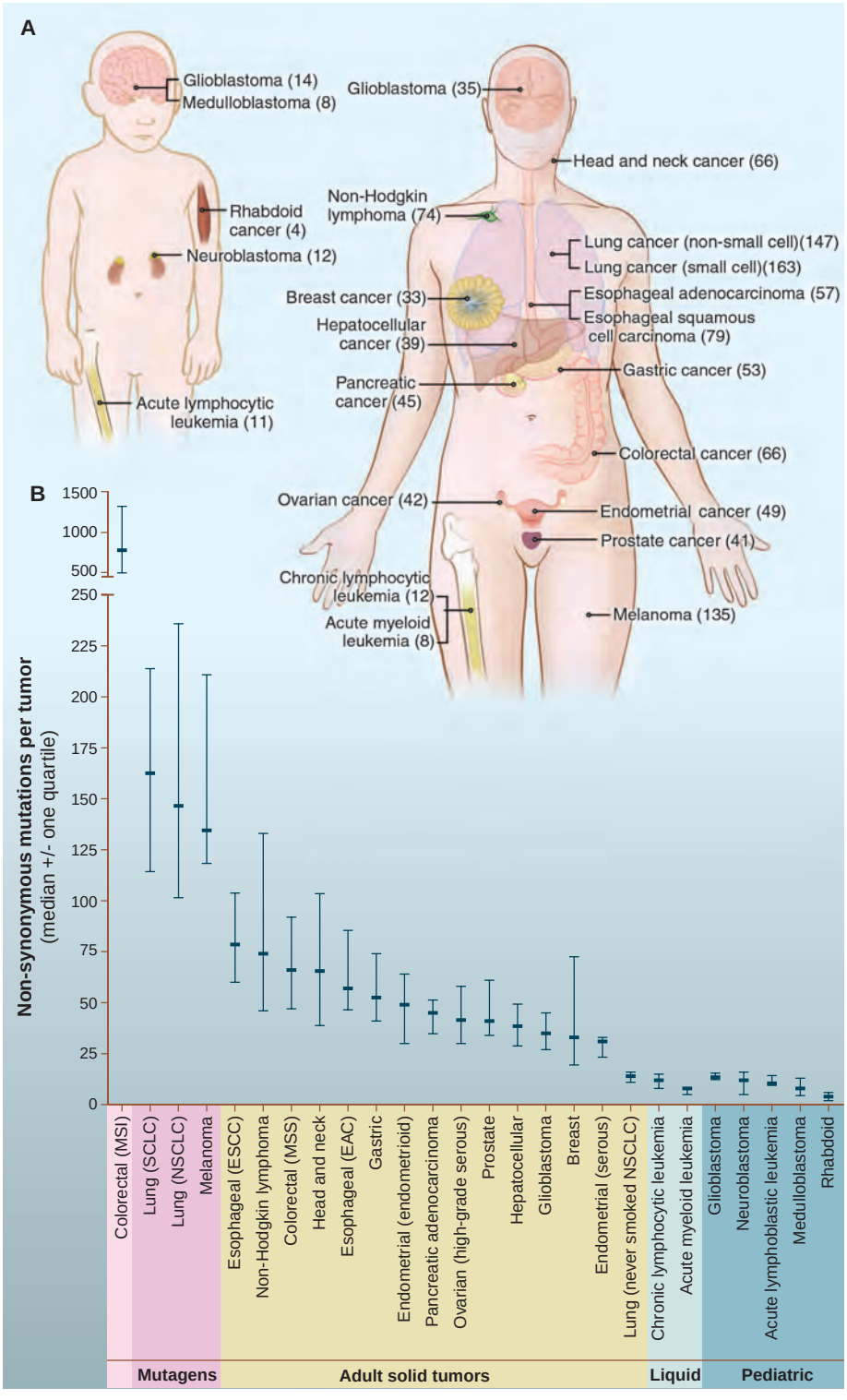

Fig. 1. Number of somatic mutations in representative human cancers, detected by genome-wide sequencing studies. (**A**) The genomes of a diverse group of adult (right) and pediatric (left) cancers have been analyzed. Numbers in parentheses indicate the median number of nonsynonymous mutations per tumor. (**B**) The median number of nonsynonymous mutations per tumor in a variety of tumor types. Horizontal bars indicate the 25 and 75% quartiles. MSI, microsatellite instability; SCLC, small cell lung cancers; NSCLC, non–small cell lung cancers; ESCC, esophageal squamous cell carcinomas; MSS, microsatellite stable; EAC, esophageal adenocarcinomas. The published data on which this figure is based are provided in table S1C.

CREDIT: FIG. 1A, E. COOK

divergence time. The number of mutations has been measured in tumors representing progressive stages of colorectal and pancreatic cancers (*11*, *16*). Applying the evolutionary clock model to these data leads to two unambiguous conclusions: First, it takes decades to develop a full-blown, metastatic cancer. Second, virtually all of the mutations in metastatic lesions were already present in a large number of cells in the primary tumors.

The timing of mutations is relevant to our understanding of metastasis, which is responsible for the death of most patients with cancer. The primary tumor can be surgically removed, but the residual metastatic lesions—often undetectable and widespread—remain and eventually enlarge, compromising the function of the lungs, liver, or other organs. From a genetics perspective, it would seem that there must be mutations that convert a primary cancer to a metastatic one, just as there are mutations that convert a normal cell to a benign tumor, or a benign tumor to a malignant one (Fig. 2). Despite intensive effort, however, consistent genetic alterations that distinguish cancers that metastasize from cancers that have not yet metastasized remain to be identified.

One potential explanation invokes mutations or epigenetic changes that are difficult to identify with current technologies (see section on "dark matter" below). Another explanation is that metastatic lesions have not yet been studied in sufficient detail to identify these genetic alterations, particularly if the mutations are heterogeneous in nature. But another possible explanation is that there are no metastasis genes. A malignant primary tumor can take many years to metastasize, but this process is, in principle, explicable by stochastic processes alone (*17*, *18*). Advanced tumors release millions of cells into the circulation each day, but these cells have short half-lives, and only a miniscule fraction establish metastatic lesions (*19*). Conceivably, these circulating cells may, in a nondeterministic manner, infrequently and randomly lodge in a capillary bed in an organ that provides a favorable microenvironment for growth. The bigger the primary tumor mass, the more likely that this process will occur. In this scenario, the continual evolution of the primary tumor would reflect local selective advantages rather than future selective advantages. The idea that growth at metastatic sites is not dependent on additional genetic alterations is also supported by recent results showing that even normal cells, when placed in suitable environments such as lymph nodes, can grow into organoids, complete with a functioning vasculature (*20*).

Other Types of Genetic Alterations in Tumors

Though the rate of point mutations in tumors is similar to that of normal cells, the rate of chromosomal changes in cancer is elevated (*21*). Therefore, most solid tumors display widespread changes in chromosome number (aneuploidy), as well as deletions, inversions, translocations,

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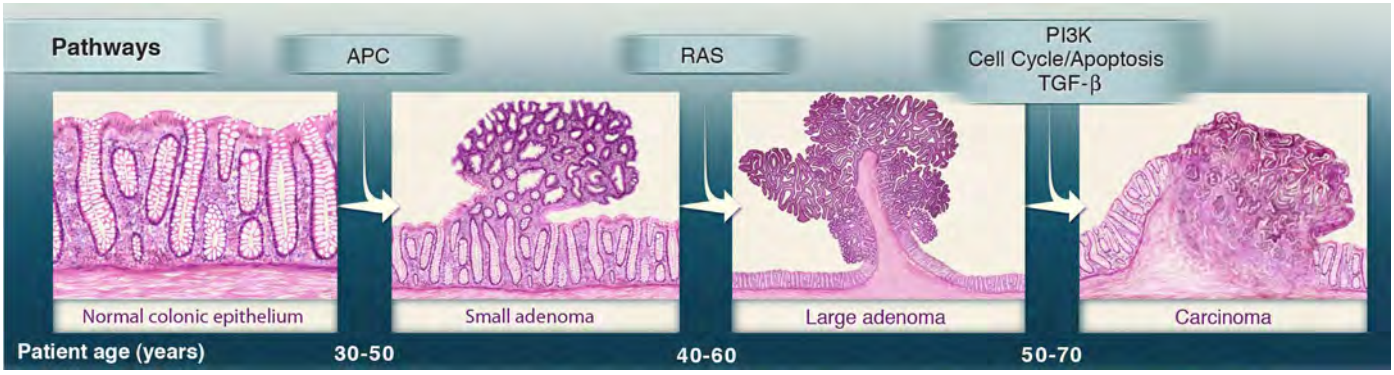

Fig. 2. Genetic alterations and the progression of colorectal cancer. The major signaling pathways that drive tumorigenesis are shown at the transitions between each tumor stage. One of several driver genes that encode components of these pathways can be altered in any individual tumor. Patient age indicates the time intervals during which the driver genes are usually mutated. Note that this model may not apply to all tumor types. TGF-β, transforming growth factor–β.

and other genetic abnormalities. When a large part of a chromosome is duplicated or deleted, it is difficult to identify the specific "target" gene(s) on the chromosome whose gain or loss confers a growth advantage to the tumor cell. Target genes are more easily identified in the case of chromosome translocations, homozygous deletions, and gene amplifications. Translocations generally fuse two genes to create an oncogene (such as *BCR-ABL* in chronic myelogenous leukemia) but, in a small number of cases, can inactivate a tumor suppressor gene by truncating it or separating it from its promoter. Homozygous deletions often involve just one or a few genes, and the target is always a tumor suppressor gene. Amplifications contain an oncogene whose protein product is abnormally active simply because the tumor cell contains 10 to 100 copies of the gene per cell, compared with the two copies present in normal cells.

Most solid tumors have dozens of translocations; however, as with point mutations, the majority of translocations appear to be passengers rather than drivers. The breakpoints of the translocations are often in "gene deserts" devoid of known genes, and many of the translocations and homozygous deletions are adjacent to fragile sites that are prone to breakage. Cancer cells can, perhaps, survive such chromosome breaks more easily than normal cells because they contain mutations that incapacitate genes like *TP53*, which would normally respond to DNA damage by triggering cell death. Studies to date indicate that there are roughly 10 times fewer genes affected by chromosomal changes than by point mutations. Figure 3 shows the types and distribution of genetic alterations that affect protein-coding genes in five representative tumor types. Protein-coding genes account for only ~1.5% of the total genome, and the number of alterations in noncoding regions is proportionately higher than the number affecting coding regions. The vast majority of the alterations in noncoding regions are presumably passengers. These noncoding mutations, as well as the numerous epigenetic changes found in cancers, will be discussed later.

Drivers Versus Passenger Mutations

Though it is easy to define a "driver gene mutation" in physiologic terms (as one conferring a selective growth advantage), it is more difficult to identify which somatic mutations are drivers and which are passengers. Moreover, it is important to point out that there is a fundamental difference between a driver gene and a driver gene mutation. A driver gene is one that contains driver gene mutations. But driver genes may also contain passenger gene mutations. For example, *APC* is a large driver gene, but only those mutations that truncate the encoded protein within its N-terminal 1600 amino acids are driver gene mutations. Missense mutations throughout the gene, as well as protein-truncating mutations in the C-terminal 1200 amino acids, are passenger gene mutations.

Numerous statistical methods to identify driver genes have been described. Some are based on the frequency of mutations in an individual gene compared with the mutation frequency of other genes in the same or related tumors after correction for sequence context and gene size (*22*, *23*). Other methods are based on the predicted effects of mutation on the encoded protein, as inferred from biophysical studies (*24*–*26*). All of these methods are useful for prioritizing genes that are most likely to promote a selective growth advantage when mutated. When the number of mutations in a gene is very high, as with *TP53* or *KRAS*, any reasonable statistic will indicate that the gene is extremely likely to be a driver gene. These highly mutated genes have been termed "mountains" (*1*). Unfortunately, however, genes with more than one, but still relatively few mutations (so called "hills") numerically dominate cancer genome landscapes (*1*). In these cases, methods based on mutation frequency and context alone cannot reliably indicate which genes are drivers, because the background rates of mutation vary so much among different patients and regions of the genome. Recent studies of normal cells have indicated that the rate of mutation varies by more than 100-fold within the genome (*27*). In tumor cells, this variation can be higher and may affect whole

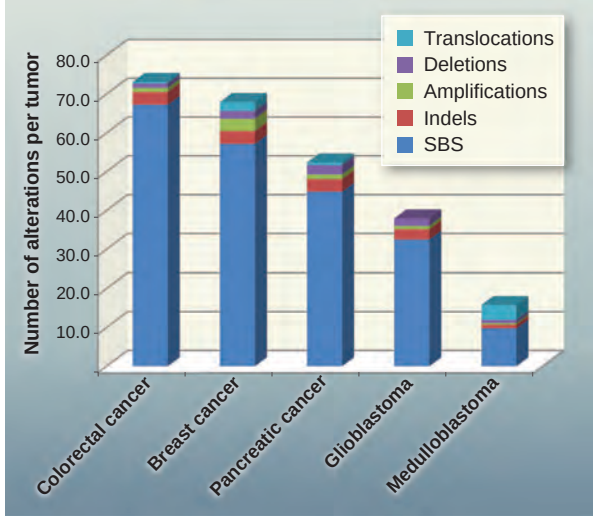

Fig. 3. Total alterations affecting protein-coding genes in selected tumors. Average number and types of genomic alterations per tumor, including single-base substitutions (SBS), small insertions and deletions (indels), amplifications, and homozygous deletions, as determined by genome-wide sequencing studies. For colorectal, breast, and pancreatic ductal cancer, and medulloblastomas, translocations are also included. The published data on which this figure is based are provided in table S1D.

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Box 1. Glossary

Adenoma: A benign tumor composed of epithelial cells.

Alternative lengthening of telomeres (ALT): A process of maintaining telomeres independent of telomerase, the enzyme normally responsible for telomere replication.

Amplification: A genetic alteration producing a large number of copies of a small segment (less than a few megabases) of the genome.

Angiogenesis: the process of forming vascular conduits, including veins, arteries, and lymphatics.

Benign tumor: An abnormal proliferation of cells driven by at least one mutation in an oncogene or tumor suppressor gene. These cells are not invasive (i.e., they cannot penetrate the basement membrane lining them), which distinguishes them from malignant cells.

Carcinoma: A type of malignant tumor composed of epithelial cells.

Clonal mutation: A mutation that exists in the vast majority of the neoplastic cells within a tumor.

Driver gene mutation (driver): A mutation that directly or indirectly confers a selective growth advantage to the cell in which it occurs.

Driver gene: A gene that contains driver gene mutations (Mut-Driver gene) or is expressed aberrantly in a fashion that confers a selective growth advantage (Epi-Driver gene).

Epi-driver gene: A gene that is expressed aberrantly in cancers in a fashion that confers a selective growth advantage.

Epigenetic: Changes in gene expression or cellular phenotype caused by mechanisms other than changes in the DNA sequence.

Exome: The collection of exons in the human genome. Exome sequencing generally refers to the collection of exons that encode proteins.

Gatekeeper: A gene that, when mutated, initiates tumorigenesis. Examples include *RB*, mutations of which initiate retinoblastomas, and *VHL*, whose mutations initiate renal cell carcinomas.

Germline genome: An individual's genome, as inherited from their parents.

Germline variants: Variations in sequences observed in different individuals. Two randomly chosen individuals differ by ~20,000 genetic variations distributed throughout the exome.

Human leukocyte antigen (HLA): A protein encoded by genes that determine an individual's capacity to respond to specific antigens or reject transplants from other individuals.

Homozygous deletion: Deletion of both copies of a gene segment (the one inherited from the mother, as well as that inherited from the father).

Indel: A mutation due to small insertion or deletion of one or a few nucleotides.

Karyotype: Display of the chromosomes of a cell on a microscopic slide, used to evaluate changes in chromosome number as well as structural alterations of chromosomes.

Kinase: A protein that catalyzes the addition of phosphate groups to other molecules, such as proteins or lipids. These proteins are essential to nearly all signal transduction pathways.

Liquid tumors: Tumors composed of hematopoietic (blood) cells, such as leukemias. Though lymphomas generally form solid masses in lymph nodes, they are often classified as liquid tumors because of their derivation from hematopoietic cells and ability to travel through lymphatics.

Malignant tumor: An abnormal proliferation of cells driven by mutations in oncogenes or tumor suppressor genes that has already invaded their surrounding stroma. It is impossible to distinguish an isolated benign tumor cell from an isolated malignant tumor cell. This distinction can be made only through examination of tissue architecture.

Metastatic tumor: A malignant tumor that has migrated away from its primary site, such as to draining lymph nodes or another organ.

Methylation: Covalent addition of a methyl group to a protein, DNA, or other molecule.

Missense mutation: A single-nucleotide substitution (e.g., C to T) that results in an amino acid substitution (e.g., histidine to arginine).

Mut-driver gene: A gene that contains driver gene mutations.

Nonsense mutation: A single-nucleotide substitution (e.g., C to T) that results in the production of a stop codon.

Nonsynonymous mutation: A mutation that alters the encoded amino acid sequence of a protein. These include missense, nonsense, splice site, translation start, translation stop, and indel mutations.

Oncogene: A gene that, when activated by mutation, increases the selective growth advantage of the cell in which it resides.

Passenger mutation (passenger): A mutation that has no direct or indirect effect on the selective growth advantage of the cell in which it occurred.

Primary tumor: The original tumor at the site where tumor growth was initiated. This can be defined for solid tumors, but not for liquid tumors.

Promoter: A region within or near the gene that helps regulate its expression.

Rearrangement: A mutation that juxtaposes nucleotides that are normally separated, such as those on two different chromosomes.

Selective growth advantage (*s*): The difference between birth and death in a cell population. In normal adult cells in the absence of injury, $s = 0.000000$.

Self-renewing tissues: Tissues whose cells normally repopulate themselves, such as those lining the gastrointestinal or urogenital tracts, as well as blood cells.

Single-base substitution (SBS): A single-nucleotide substitution (e.g., C to T) relative to a reference sequence or, in the case of somatic mutations, relative to the germline genome of the person with a tumor.

Solid tumors: Tumors that form discrete masses, such as carcinomas or sarcomas.

Somatic mutations: Mutations that occur in any non–germ cell of the body after conception, such as those that initiate tumorigenesis.

Splice sites: Small regions of genes that are juxtaposed to the exons and direct exon splicing.

Stem cell: An immortal cell that can repopulate a particular cell type.

Subclonal mutation: A mutation that exists in only a subset of the neoplastic cells within a tumor.

Translocation: A specific type of rearrangement where regions from two nonhomologous chromosomes are joined.

Tumor suppressor gene: A gene that, when inactivated by mutation, increases the selective growth advantage of the cell in which it resides.

Untranslated regions: Regions within the exons at the 5′ and 3′ ends of the gene that do not encode amino acids.

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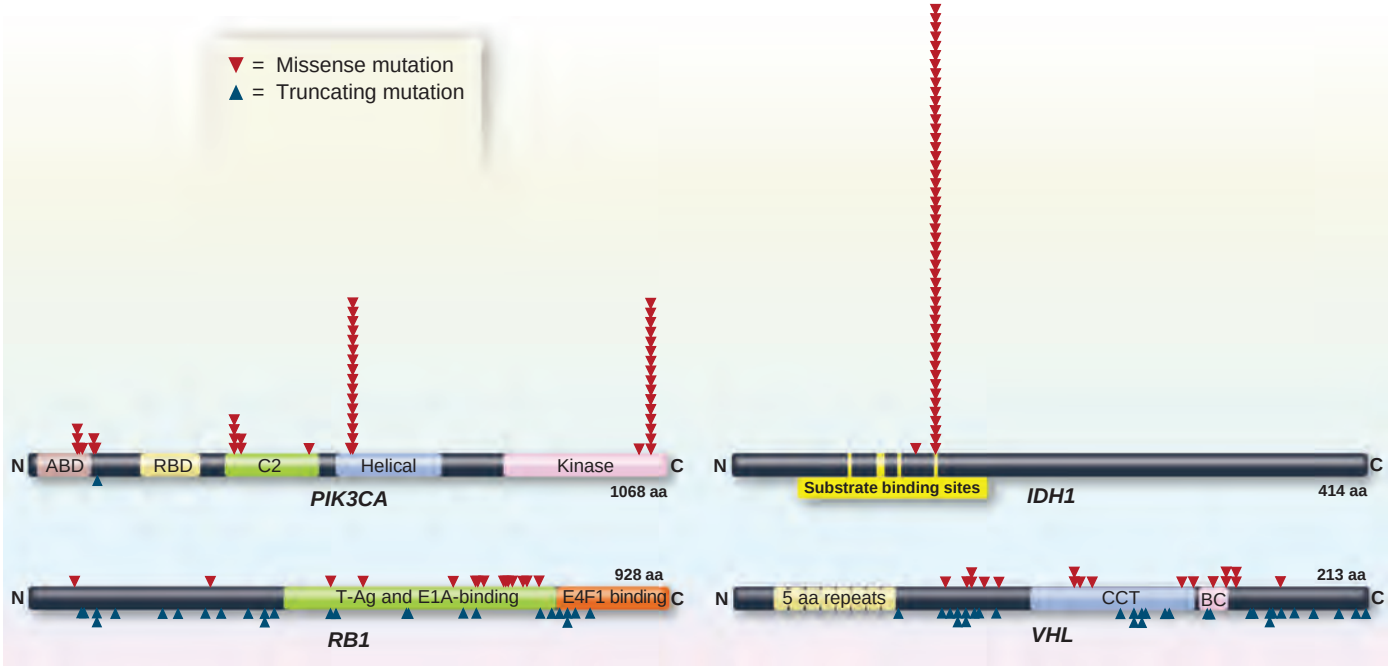

Fig. 4. Distribution of mutations in two oncogenes (*PIK3CA* and *IDH1*) and two tumor suppressor genes (*RB1* and *VHL*). The distribution of missense mutations (red arrowheads) and truncating mutations (blue arrowheads) in representative oncogenes and tumor suppressor genes are shown. The data were collected from genome-wide studies annotated in the COSMIC database (release version 61). For *PIK3CA* and *IDH1*, mutations obtained from the COSMIC database were randomized by the Excel RAND function, and the first 50 are shown. For *RB1* and *VHL*, all mutations recorded in COSMIC are plotted. aa, amino acids.

regions of the genome in an apparently random fashion (*28*). Thus, at best, methods based on mutation frequency can only prioritize genes for further analysis but cannot unambiguously identify driver genes that are mutated at relatively low frequencies.

Further complicating matters, there are two distinct meanings of the term "driver gene" that are used in the cancer literature. The driver-versus-passenger concept was originally used to distinguish mutations that caused a selective growth advantage from those that did not (*29*). According to this definition, a gene that does not harbor driver gene mutations cannot be a driver gene. But many genes that contain few or no driver gene mutations have been labeled driver genes in the literature. These include genes that are overexpressed, underexpressed, or epigenetically altered in tumors, or those that enhance or inhibit some aspect of tumorigenicity when their expression is experimentally manipulated. Though a subset of these genes may indeed play an important role in the neoplastic process, it is confusing to lump them all together as driver genes.

To reconcile the two connotations of driver genes, we suggest that genes suspected of increasing the selective growth advantage of tumor cells be categorized as either "Mut-driver genes" or "Epi-driver genes." Mut-driver genes contain a sufficient number or type of driver gene mutations to unambiguously distinguish them from other genes. Epi-driver genes are expressed aberrantly in tumors but not frequently mutated; they are altered through changes in DNA methylation or chromatin modification that persist as the tumor cell divides.

A Ratiometric Method to Identify and Classify Mut-Driver Genes

If mutation frequency, corrected for mutation context, gene length, and other parameters, cannot reliably identify modestly mutated driver genes, what can? In our experience, the best way to identify Mut-driver genes is through their pattern of mutation rather than through their mutation frequency. The patterns of mutations in well-studied oncogenes and tumor suppressor genes are highly characteristic and nonrandom. Oncogenes are recurrently mutated at the same amino acid positions, whereas tumor suppressor genes are mutated through protein-truncating alterations throughout their length (Fig. 4 and table S2A).

On the basis of these mutation patterns rather than frequencies, we can determine which of the 18,306 mutated genes containing a total of 404,863 subtle mutations that have been recorded in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (*30*) are Mut-driver genes and whether they are likely to function as oncogenes or tumor suppressor genes. To be classified as an oncogene, we simply require that >20% of the recorded mutations in the gene are at recurrent positions and are missense (see legend to table S2A). To be classified as a tumor suppressor gene, we analogously require that >20% of the recorded mutations in the gene are inactivating. This "20/20 rule" is lenient in that all well-documented cancer genes far surpass these criteria (table S2A).

The following examples illustrate the value of the 20/20 rule. When *IDH1* mutations were first identified in brain tumors, their role in tumorigenesis was unknown (*2*, *31*). Initial functional studies suggested that *IDH1* was a tumor suppressor gene and that mutations inactivated this gene (*32*). However, nearly all of the mutations in *IDH1* were at the identical amino acid, codon 132 (Fig. 4). As assessed by the 20/20 rule, this distribution unambiguously indicated that *IDH1* was an oncogene rather than a tumor suppressor gene, and this conclusion was eventually supported by biochemical experiments (*33*, *34*). Another example is provided by mutations in *NOTCH1*. In this case, some functional studies suggested that *NOTCH1* was an oncogene, whereas others suggested it was a tumor suppressor gene (*35*, *36*). The situation could be clarified through the application of the 20/20 rule to *NOTCH1* mutations in cancers. In "liquid tumors" such as lymphomas and leukemias, the mutations were often recurrent and did not truncate the predicted protein (*37*). In squamous cell carcinomas, the mutations were not recurrent and were usually inactivating (*38*–*40*). Thus, the genetic data clearly indicated that *NOTCH1* functions differently in different tumor types. The idea that the same gene can function

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in completely opposite ways in different cell types is important for understanding cell signaling pathways.

How Many Mut-Driver Genes Exist?

Though all 20,000 protein-coding genes have been evaluated in the genome-wide sequencing studies of 3284 tumors, with a total of 294,881 mutations reported, only 125 Mut-driver genes, as defined by the 20/20 rule, have been discovered to date (table S2A). Of these, 71 are tumor suppressor genes and 54 are oncogenes. An important but relatively small fraction (29%) of these genes was discovered to be mutated through unbiased genome-wide sequencing; most of these genes had already been identified by previous, more directed investigations.

How many more Mut-driver genes are yet to be discovered? We believe that a plateau is being reached, because the same Mut-driver genes keep being "rediscovered" in different tumor types. For example, *MLL2* and *MLL3* mutations were originally discovered in medulloblastomas (*41*) and were subsequently discovered to be mutated in non-Hodgkin lymphomas, prostate cancers, breast cancers, and other tumor types (*42*–*45*). Similarly, *ARID1A* mutations were first discovered to be mutated in clear-cell ovarian cancers (*46*, *47*) and were subsequently shown to be mutated in tumors of several other organs, including those of the stomach and liver (*48*–*50*). In recent studies of several types of lung cancer (*4*, *51*, *52*), nearly all genes found to be mutated at significant frequencies had already been identified in tumors of other organs. In other words, the number of frequently altered Mut-driver genes (mountains) is nearing saturation. More mountains will undoubtedly be discovered, but these will likely be in uncommon tumor types that have not yet been studied in depth.

The newly discovered Mut-driver genes that have been detected through genome-wide sequencing have often proved illuminating. For example, nearly half of these genes encode proteins that directly regulate chromatin through modification of histones or DNA. Examples include the histones HIST1H3B and H3F3A, as well as the proteins DNMT1 and TET1, which covalently modify DNA, EZH2, SETD2, and KDM6A, which, in turn, methylate or demethylate histones (*53*–*57*). These discoveries have profound implications for understanding the mechanistic basis of the epigenetic changes that are rampant in tumors (*58*). The discovery of genetic alterations in genes encoding mRNA splicing factors, such as *SF3B1* and *U2AF1* (*59*–*61*), was similarly stunning, as mutations in these genes would be expected to lead to a plethora of nonspecific cellular stresses rather than to promote specific tumor types. Another example is provided by mutations in the cooperating proteins ATRX and DAXX (*62*). Tumors with mutations in these genes all have a specific type of telomere elongation process termed "ALT" (for "alternative lengthening of telomeres") (*63*). Though the ALT phenotype had been recognized for more than a decade, its genetic basis was mysterious before the discovery of mutations of these genes and their perfect correlation with the ALT phenotype (*64*). A final example is provided by *IDH1* and *IDH2*, whose mutations have stimulated the burgeoning field of tumor metabolism (*65*) and have had fascinating implications for epigenetics (*66*, *67*).

The Mut-driver genes listed in table S2A are affected by subtle mutations: base substitutions, intragenic insertions, or deletions. As noted above, Mut-driver genes can also be altered by less subtle changes, such as translocations, amplifications, and large-scale deletions. As with point mutations, it can be difficult to distinguish Mut-driver genes that are altered by these types of changes from genes that contain only passenger mutations. Genes that are not point-mutated, but are recurrently amplified (e.g., *MYC* family genes) or homozygously deleted (e.g., *MAP2K4*) and that meet other criteria (e.g., being the only gene in the amplicon or homozygously deleted region) are listed in table S2B. This adds 13 Mut-driver genes—10 oncogenes that are amplified and 3 tumor suppressor genes that are homozygously deleted—to the 125 driver genes that are affected by subtle mutations, for a total of 138 driver genes discovered to date (table S2).

Translocations provide similar challenges for driver classification. An important discovery related to this point is chromothripsis (*68*), a rare cataclysmic event involving one or a small number of chromosomes that results in a large number of chromosomal rearrangements. This complicates any inferences about causality, in the same way that mismatch repair deficiency compromises the interpretation of point mutations. However, for completeness, all fusion genes that have been identified in at least three independent tumors are listed in table S3. Virtually all of these genes were discovered through conventional approaches before the advent of genome-wide DNA sequencing studies, with some notable exceptions such as those described in (*6*) and (*69*). The great majority of these translocations are found in liquid tumors (leukemias and lymphomas) (table S3C) or mesenchymal tumors (table S3B) and were initially identified through karyotypic analyses. A relatively small number of recurrent fusions, the most important of which include *ERG* in prostate cancers (*70*) and *ALK* in lung cancers (*71*), have been described in more common tumors (table S3A).

Genes exist that predispose to cancer when inherited in mutant form in the germ line, but are not

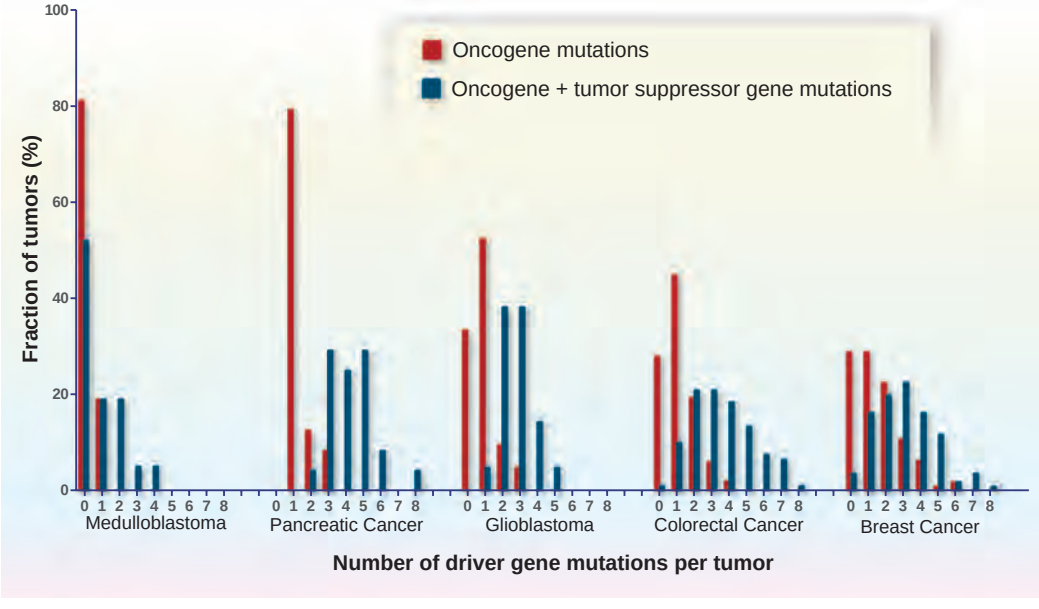

Fig. 5. Number and distribution of driver gene mutations in five tumor types. The total number of driver gene mutations [in oncogenes and tumor suppressor genes (TSGs)] is shown, as well as the number of oncogene mutations alone. The driver genes are listed in tables S2A and S2B. Translocations are not included in this figure, because few studies report translocations along with the other types of genetic alterations on a per-case basis. In the tumor types shown here, translocations affecting driver genes occur in less than 10% of samples. The published data on which this figure is based are provided in table S1E.

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somatically mutated in cancer to a substantial degree. These genes generally do not confer an increase in selective growth advantage when they are abnormal, but they stimulate tumorigenesis in indirect ways (such as by increasing genetic instability, as discussed later in this Review). For completeness, these genes and the hereditary syndromes for which they are responsible are listed in table S4.

Dark Matter

Classic epidemiologic studies have suggested that solid tumors ordinarily require five to eight "hits," now interpreted as alterations in driver genes, to develop (*72*). Is this number compatible with the molecular genetic data? In pediatric tumors such as medulloblastomas, the number of driver gene mutations is low (zero to two), as expected from the discussion above (Fig. 5). In common adult tumors—such as pancreatic, colorectal, breast, and brain cancers—the number of mutated driver genes is often three to six, but several tumors have only one or two driver gene mutations (Fig. 5). How can this be explained, given the widely accepted notion that tumor development and progression require multiple, sequential genetic alterations acquired over decades?

First, technical issues explain some of the "missing mutations." Genome-wide sequencing is far from perfect, at least with the technologies available today. Some regions of the genome are not well represented because their sequences are difficult to amplify, capture, or unambiguously map to the genome (*73*–*76*). Second, there is usually a wide distribution in the number of times that a specific nucleotide in a given gene is observed in the sequence data, so some regions will not be well represented by chance factors alone (*77*). Finally, primary tumors contain not only neoplastic cells, but also stromal cells that dilute the signal from the mutated base, further reducing the probability of finding a mutation (*78*).

What fraction of mutations are missed by these three technical issues? A recent study of pancreatic cancers is informative in this regard. Biankin *et al*. used immunohistochemical and genetic analyses to select a set of primary tumor samples enriched in neoplastic cells (*79*). They used massively parallel sequencing to analyze the exomes of these samples, then compared their mutational data with a set of pancreatic cancer cell lines and xenografts in which mutations had previously been identified, using conventional Sanger sequencing, and confirmed to be present in the primary tumors (*3*, *16*). Only 159 (63%) of the expected 251 driver gene mutations were identified in the primary tumors studied by next-generation sequencing alone, indicating a false-negative rate of 37%. Genome-wide studies in which the proportion of neoplastic cells within tumors is not as carefully evaluated as in (*79*) will have higher false-negative rates. Moreover, these technical problems are exacerbated in whole-genome studies compared with exomic analyses, because the sequence coverage of the former is often lower than that of the latter (generally 30-fold in whole-genome studies versus more than 100-fold in exomic studies).

Conceptual issues also limit the number of detectable drivers. Virtually all studies, either at the whole-genome or whole-exome level, have focused on the coding regions. The reason for this is practical; it is difficult enough to identify driver gene mutations when they qualitatively alter the sequence of the encoded protein. Trying to make sense of intergenic or intronic mutations is much more difficult. Based on analogous studies of the identifiable mutations in patients with monogenic diseases, more than 80% of mutations should be detectable through analysis of the coding regions (*80*). However, this still leaves some mutations as unidentifiable "dark matter," even in the germline genomes of heritable cases, which are usually easier to interpret than the somatic mutations in cancers. The first examples of light coming to such dark matter have recently been published: Recurrent mutations in the promoter of the *TERT* gene, encoding the catalytic subunit of telomerase, have been identified and shown to activate its transcription (*81*, *82*).

Mut-driver genes other than those listed in table S2 will undoubtedly be discovered as genome-wide sequencing continues. However, based on the trends noted above, most of the Mut-driver genes will likely be mountains in rare tumor types or small hills in common tumor types; thus, these genes are unlikely to account for the bulk of the presumptive dark matter. Other types of dark matter can be envisioned, however. Copy-number alterations are ubiquitous in cancers, at either the whole-chromosome or subchromosomal levels. These alterations could subtly change the expression of their driver genes. Recent studies have suggested that the loss of one copy of chromosomes containing several tumor suppressor genes, each plausibly connected to neoplasia but not altered by

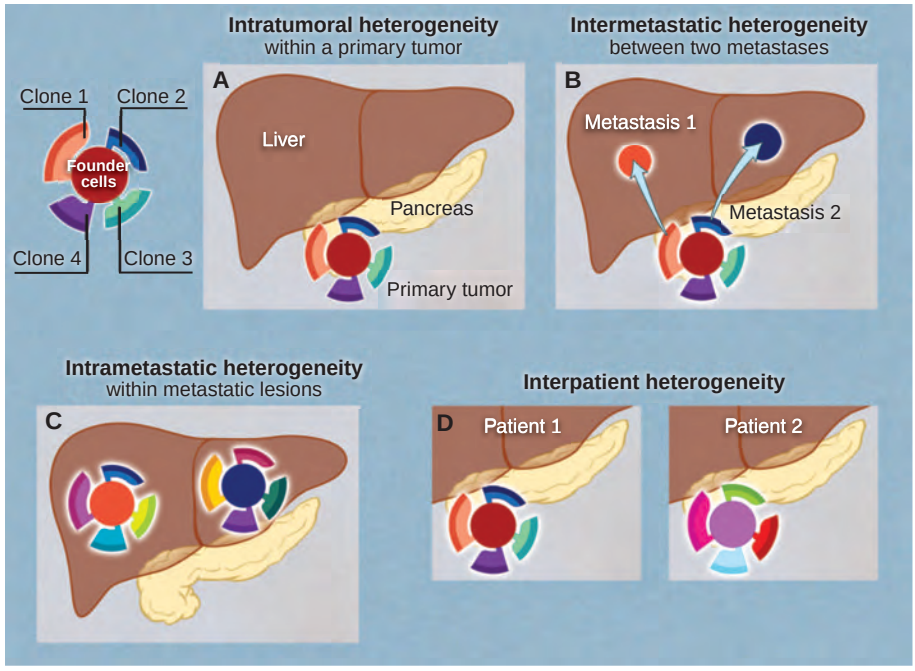

Fig. 6. Four types of genetic heterogeneity in tumors, illustrated by a primary tumor in the pancreas and its metastatic lesions in the liver. Mutations introduced during primary tumor cell growth result in clonal heterogeneity. At the top left, a typical tumor is represented by cells with a large fraction of the total mutations (founder cells) from which subclones are derived. The differently colored regions in the subclones represent stages of evolution within a subclone. (**A**) Intratumoral: heterogeneity among the cells of the primary tumor. (**B**) Intermetastatic: heterogeneity among different metastatic lesions in the same patient. In the case illustrated here, each metastasis was derived from a different subclone. (**C**) Intrametastatic: heterogeneity among the cells of each metastasis develops as the metastases grow. (**D**) Interpatient: heterogeneity among the tumors of different patients. The mutations in the founder cells of the tumors of these two patients are almost completely distinct (see text).

CREDIT: FIG. 6, E. COOK

mutation, may confer a selective growth advantage (*83*, *84*).

The most obvious source of dark matter is in Epi-driver genes. Human tumors contain large numbers of epigenetic changes affecting DNA or chromatin proteins. For example, a recent study of colorectal cancers showed that more than 10% of the protein-coding genes were differentially methylated when compared with normal colorectal epithelial cells (*85*). Some of these changes (i.e., those in Epi-driver genes) are likely to provide a selective growth advantage (*86*, *87*). For example, epigenetic silencing of *CDK2NA* and *MLH1* is much more common than mutational inactivation of either of these two well-recognized driver genes (*85*) However, there is a critical difference between a genetic and an epigenetic change in a gene. Unlike the sequence of a gene in a given individual, methylation is plastic, varying with cell type, developmental stage, and patient age (*21*). The methylation state of the normal precursor cells that initiate tumorigenesis is unknown; these cells, such as normal stem cells, may represent only a tiny fraction of the cells in a normal organ. This plasticity also means that methylation can change under microenvironmental cues, such as those associated with low nutrient concentrations or abnormal cell contacts. It is therefore difficult to know whether specific epigenetic changes observed in cancer cells reflect, rather than contribute to, the neoplastic state. Criteria for distinguishing epigenetic changes that exert a selective growth advantage from those that do not (passenger epigenetic changes) have not yet been formulated. Given that Epi-driver genes are likely to compose a major component of the dark matter, further research on this topic is essential (*58*).

Genetic Heterogeneity

The mutations depicted in Fig. 1 are clonal; that is, they are present in the majority of the neoplastic cells in the tumors. But additional, subclonal (i.e., heterogeneous within the tumor) mutations are important for understanding tumor evolution. Four types of genetic heterogeneity are relevant to tumorigenesis (Fig. 6):

1) Intratumoral: heterogeneity among the cells of one tumor. This type of heterogeneity has been recognized for decades. For example, it is rare to see a cytogenetic study of a solid tumor in which all of the tumor cells display the same karyotype (*88*). The same phenomenon has been noted for individual genes [e.g., (*89*)] and more recently has been observed throughout the genome (*16*, *90*–*96*). This kind of heterogeneity must exist: Every time a normal (or tumor) cell divides, it acquires a few mutations, and the number of mutations that distinguish any two cells simply marks the time from their last common ancestor (their founder cell). Cells at the opposite ends of large tumors will be spatially distinct and, in general, will display more differences than neighboring cells (*16*). This phenomenon is analogous to speciation, wherein organisms on different islands are more likely to diverge from one another than are organisms on the same island.

In studies that have evaluated intratumoral heterogeneity by genome-wide sequencing, the majority of somatic mutations are present in all tumor cells. These mutations form the trunk of the somatic evolutionary tree. What is the importance of the mutations in the branches (i.e., those that are not shared by all tumor cells)? From a medical perspective, these mutations are often meaningless because the primary tumors are surgically removed. How much heterogeneity existed in the various branches before surgery is not important. However, this heterogeneity provides the seeds for intermetastastic heterogeneity, which is of great clinical importance.

2) Intermetastatic: heterogeneity among different metastatic lesions of the same patient. The vast majority of cancer patients die because their tumors were not removed before metastasis to surgically inaccessible sites, such as the liver, brain, lung, or bone. Patients who relapse with a single metastatic lesion can often still be cured by surgery or radiotherapy, but single metastases are the exception rather than the rule. A typical patient on a clinical trial has a dozen or more metastatic lesions large enough to be visualized by imaging, and many more that are smaller. If each of the metastatic lesions in a single patient was founded by a cell with a very different genetic constitution, then chemotherapeutic cures would be nearly impossible to achieve: Eradicating a subset of the metastatic lesions in a patient will not be adequate for long-term survival.

How much heterogeneity is there among different metastatic lesions? In short, a lot. It is not uncommon for one metastatic lesion to have 20 clonal genetic alterations not shared by other metastases in the same patient (*16*, *97*). Because they are clonal, these mutations occurred in the founder cell of the metastasis; that is, the cell that escaped from the primary tumor and multiplied to form the metastasis. The founder cell for each metastasis is present in different, geographically distinct areas of the primary tumors, as expected (*16*).

This potentially disastrous situation is tempered by the fact that the heterogeneity appears largely confined to passenger gene mutations. In most of the studies documenting heterogeneity in malignancies, the Mut-driver genes are present in the trunks of the trees, though exceptions have been noted (*95*). These findings are consistent with the idea, discussed above, that the genetic alterations required for metastasis were present (i.e., selected for) before metastasis actually occurred. The data are also consistent with the observation that in patients responsive to targeted agents, the response is often seen in all metastatic lesions rather than just a small subset (*98*).

3) Intrametastatic: heterogeneity among the cells of an individual metastasis. Each metastasis is established by a single cell (or small group of cells) with a set of founder mutations. As it grows, the metastasis acquires new mutations with each cell division. Though the founder mutations may make the lesion susceptible to antitumor agents, the new mutations provide the seeds for drug resistance. Unlike primary tumors, the metastatic lesions generally cannot be removed by surgery and must be treated with systemic therapies. Patients with complete responses to targeted therapies invariably relapse. Most of the initial lesions generally recur, and the time frame at which they recur is notably similar. This time course can be explained by the presence of resistance mutations that existed within each metastasis before the onset of the targeted therapy (*99*–*102*). Calculations show that any metastatic lesion of a size visible on medical imaging has thousands of cells (among the billions present) that are already resistant to virtually any drug that can be imagined (*99*, *101*, *102*). Thus, recurrence is simply a matter of time, entirely predictable on the basis of known mutation frequencies and tumor cell growth rates. This "fait accompli" can be circumvented, in principle, by treatment with multiple agents, as it is unlikely that a single tumor cell will be resistant to multiple drugs that act on different targets.

4) Interpatient: heterogeneity among the tumors of different patients. This type of heterogeneity has been observed by every oncologist; no two cancer patients have identical clinical courses, with or without therapy. Some of these differences could be related to host factors, such as germline variants that determine drug half-life or vascular permeability to drugs or cells, and some could be related to nongenetic factors (*103*). However, much of this interpatient heterogeneity is probably related to somatic mutations within tumors. Though several dozen somatic mutations may be present in the breast cancers from two patients, only a small number are in the same genes, and in the vast majority of cases, these are the Mut-driver genes (*1*, *104*, *105*). Even in these driver genes, the actual mutations are often different. Mutations altering different domains of a protein would certainly not be expected to have identical effects on cellular properties, as experimentally confirmed (*106*). Though it may seem that different mutations in adjacent codons would have identical effects, detailed studies of large numbers of patients have shown that this need not be the case. For example, a $Gly^{12} \rightarrow Asp^{12}$ (G12D) mutation of *KRAS* does not have the same clinical implications as a G13D mutation of the same gene (*107*). Interpatient heterogeneity has always been one of the major obstacles

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to designing uniformly effective treatments for cancer. Efforts to individualize treatments based on knowledge of the genomes of cancer patients are largely based on an appreciation of this heterogeneity.

Signaling Pathways in Tumors

The immense complexity of cancer genomes that could be inferred from the data described above is somewhat misleading. After all, even advanced tumors are not completely out of control, as evidenced by the dramatic responses to agents that target mutant *BRAF* in melanomas (*108*) or mutant *ALK* in lung cancers (*109*). Albeit transient, these responses mean that interference with even a single mutant gene product is sufficient to stop cancer in its tracks, at least transiently. How can the genomic complexity of cancer be reconciled with these clinical observations?

Two concepts bear on this point. The first, mentioned above, is that >99.9% of the alterations in tumors (including point mutations, copy-number alterations, translocations, and epigenetic changes distributed throughout the genome, not just in the coding regions) are immaterial to neoplasia. They are simply passenger changes that mark the time that has elapsed between successive clonal expansions. Normal cells also undergo genetic alterations as they divide, both at the nucleotide and chromosomal levels. However, normal cells are programmed to undergo cell death in response to such alterations, perhaps as a protective mechanism against cancer. In contrast, cancer cells have evolved to tolerate genome complexity by acquiring mutations in genes such as *TP53* (*110*). Thus, genomic complexity is, in part, the result of cancer, rather than the cause.

To appreciate the second concept, one must take the 30,000-foot view. A jungle might look chaotic at ground level, but the aerial view shows a clear order, with all the animals gathering at the streams at certain points in the day, and all the streams converging at a river. There is order in cancer, too. Mutations in all of the 138 driver genes listed in table S2 do one thing: cause a selective growth advantage, either directly or indirectly. Moreover, there appears to be only a limited number of cellular signaling pathways through which a growth advantage can be incurred (Fig. 7 and table S5).

All of the known driver genes can be classified into one or more of 12 pathways (Fig. 7). The discovery of the molecular components of these pathways is one of the greatest achievements of biomedical research, a tribute to investigators working in fields that encompass biochemistry, cell biology, and development, as well as cancer. These pathways can themselves be further organized into three core cellular processes:

1) Cell fate: Numerous studies have demonstrated the opposing relationship between cell division and differentiation, the arbiters of cell fate. Dividing cells that are responsible for populating normal tissues (stem cells) do not differentiate, and vice versa. Regenerative medicine is based on this distinction, predicated on ways to get differentiated cells to dedifferentiate into stem cells, then forcing the stem cells to differentiate into useful cell types for transplantation back into the patient. Many of the genetic alterations in cancer abrogate the precise balance between differentiation and division, favoring the latter. This causes a selective growth advantage, because differentiating cells eventually die or become quiescent. Pathways that function through this process include APC, HH, and NOTCH, all of which are well known to control cell fate in organisms ranging from worms to mammals (*111*). Genes encoding chromatin-modifying enzymes can also be included in this category. In normal development, the heritable switch from division to differentiation is not determined by mutation, as it is in cancer, but rather by epigenetic alterations affecting DNA and chromatin proteins. What better way to subvert this normal mechanism for controlling tissue architecture than to debilitate the epigenetic modifying apparatus itself?

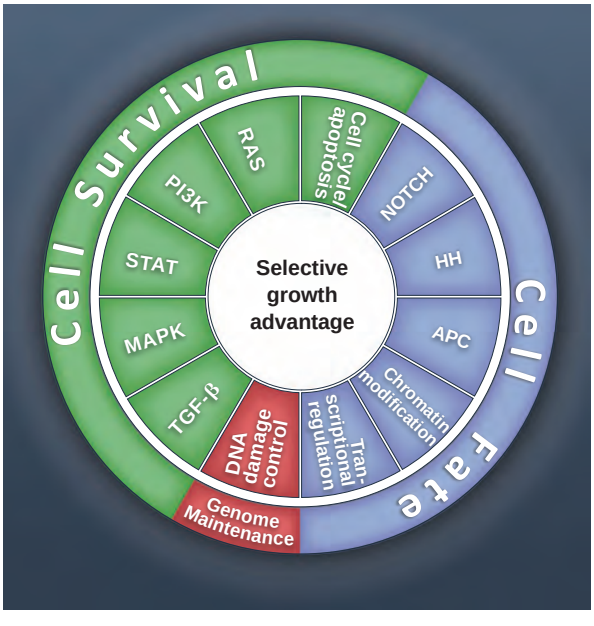

Fig. 7. Cancer cell signaling pathways and the cellular processes they regulate. All of the driver genes listed in table S2 can be classified into one or more of 12 pathways (middle ring) that confer a selective growth advantage (inner circle; see main text). These pathways can themselves be further organized into three core cellular processes (outer ring). The publications on which this figure is based are provided in table S5.

2) Cell survival: Though cancer cells divide abnormally because of cell-autonomous alterations, such as those controlling cell fate, their surrounding stromal cells are perfectly normal and do not keep pace. The most obvious ramification of this asymmetry is the abnormal vasculature of tumors. As opposed to the well-ordered network of arteries, veins, and lymphatics that control nutrient concentrations in normal tissues, the vascular system in cancers is tortuous and lacks uniformity of structure (*112*, *113*). Normal cells are always within 100 μm of a capillary, but this is not true for cancer cells (*114*). As a result, a cancer cell acquiring a mutation that allows it to proliferate under limiting nutrient concentrations will have a selective growth advantage, thriving in environments in which its sister cells cannot. Mutations of this sort occur, for example, in the *EGFR*, *HER2*, *FGFR2*, *PDGFR*, *TGFβR2*, *MET*, *KIT*, *RAS*, *RAF*, *PIK3CA*, and *PTEN* genes (table S2A). Some of these genes encode receptors for the growth factors themselves, whereas others relay the signal from the growth factor to the interior of the cell, stimulating growth when activated (*115*, *116*). For instance, mutations in *KRAS* or *BRAF* genes confer on cancer cells the ability to grow in glucose concentrations that are lower than those required for the growth of normal cells or of cancer cells that do not have mutations in these genes (*117*, *118*). Progression through the cell cycle (and its antithesis, apoptosis) can be directly controlled by intracellular metabolites, and driver genes that directly regulate the cell cycle or apoptosis, such as *CDKN2A*, *MYC*, and *BCL2*, are often mutated in cancers. Another gene whose mutations enhance cell survival is *VHL*, the product of which stimulates angiogenesis through the secretion of vascular endothelial growth factor. What better way to provision growth factors to a rogue tumor than to lure the unsuspecting vasculature to its hideout?

3) Genome maintenance: As a result of the exotic microenvironments in which they reside, cancer cells are exposed to a variety of toxic substances, such as reactive oxygen species. Even without microenvironmental poisons, cells make mistakes while replicating their DNA or during division (*119*, *120*), and checkpoints exist to either slow down such cells or make them commit suicide (apoptosis) under such circumstances (*110*, *121*, *122*). Although it is good for the organism to remove these damaged cells, tumor cells that can survive the damage will, by definition, have a selective growth advantage. Therefore, it is not surprising that genes whose mutations abrogate these checkpoints, such as *TP53* and *ATM*, are mutated in cancers

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(*123*). Defects in these genes can also indirectly confer a selective growth advantage by allowing cells that have a gross chromosomal change favoring growth, such as a translocation or an extra chromosome, to survive and divide. Analogously, genes that control point mutation rates, such as *MLH1* or *MSH2*, are mutated in cancers (table S2A) or in the germ line of patients predisposed to cancers (table S4) because they accelerate the acquisition of mutations that function through processes that regulate cell fate or survival. What better way to promote cancer than by increasing the rate of occurrence of the mutations that drive the process?

Because the protein products of genes regulating cell fate, cell survival, and genome maintenance often interact with one another, the pathways within them overlap; they are not as discrete as might be inferred from the description above. However, grouping genes into pathways makes perfect sense from a genetics standpoint. Given that cancer is a genetic disease, the principles of genetics should apply to its pathogenesis. When performing a conventional mutagenesis screen in bacteria, yeast, fruit flies, or worms, one expects to discover mutations in several different genes that confer similar phenotypes. The products of these genes often interact with one another and define a biochemical or developmental pathway. Therefore, it should not be surprising that several different genes can result in the same selective growth advantage for cancer cells and that the products of these genes interact. The analogy between cancer pathways and biochemical or developmental pathways in other organisms goes even deeper: The vast majority of our knowledge of the function of driver genes has been derived from the study of the pathways through which their homologs work in nonhuman organisms. Though the functions are not identical to those in human cells, they are highly related and have provided the starting point for analogous studies in human cells.

Recognition of these pathways also has important ramifications for our ability to understand interpatient heterogeneity. One lung cancer might have an activating mutation in a receptor for a stimulatory growth factor, making it able to grow in low concentrations of epidermal growth factor (EGF). A second lung cancer might have an activating mutation in *KRAS*, whose protein product normally transmits the signal from the epidermal growth factor receptor (EGFR) to other cell signaling molecules. A third lung cancer might have an inactivating mutation in *NF1*, a regulatory protein that normally inactivates the KRAS protein. Finally, a fourth lung cancer might have a mutation in *BRAF*, which transmits the signal from KRAS to downstream kinases (Fig. 8). One would predict that mutations in the various components of a single pathway would be mutually exclusive—that is, not occurring in the same tumor—and this has been experimentally confirmed (*124*, *125*). Apart from being intellectually satisfying, knowledge of these pathways has implications for cancer therapy, as discussed in the next section.

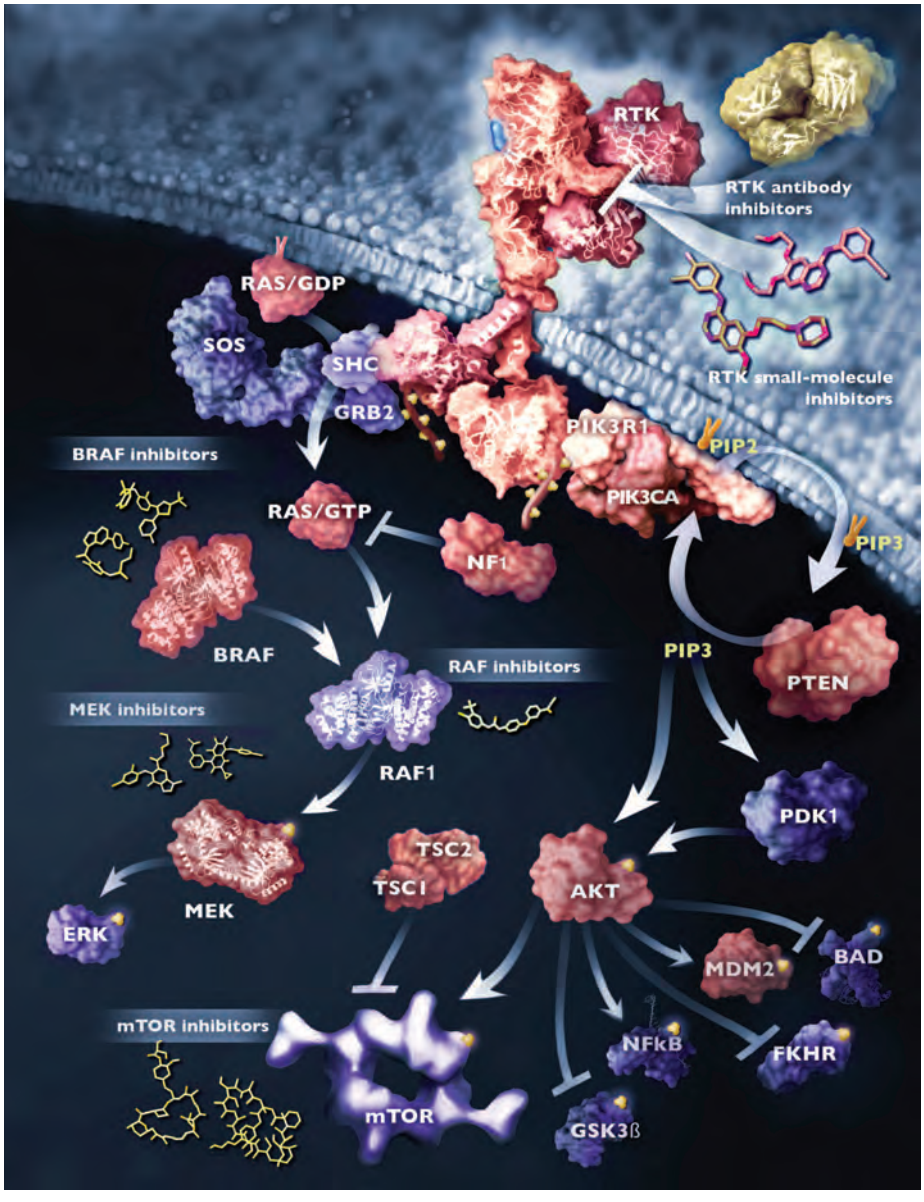

Fig. 8. Signal transduction pathways affected by mutations in human cancer. Two representative pathways from Fig. 7 (RAS and PI3K) are illustrated. The signal transducers are color coded: red indicates protein components encoded by the driver genes listed in table S2; yellow balls denote sites of phosphorylation. Examples of therapeutic agents that target some of the signal transducers are shown. RTK, receptor tyrosine kinase; GDP, guanosine diphosphate; MEK, MAPK kinase; ERK, extracellular signal–regulated kinase; NFkB, nuclear factor κB; mTOR, mammalian target of rapamycin.

CREDIT: FIG. 8, A. DIXON

A Perspective on Genome-Based Medicine in Oncology

Opportunities

Though cancer genome sequencing is a relatively new endeavor, it has already had an impact on the clinical care of cancer patients. The recognition that certain tumors contain activating mutations in driver genes encoding protein kinases has led to the development of small-molecule inhibitor drugs targeting those kinases.

Representative examples of this type of genome-based medicine include the use of EGFR kinase inhibitors to treat cancers with *EGFR* gene mutations (*126*), the aforementioned anaplastic lymphoma kinase (ALK) inhibitors to treat cancers with *ALK* gene translocations (*109*), and specific inhibitors of mutant BRAF

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to treat cancers with *BRAF* mutations (*108*). Before instituting treatment with such agents, it is imperative to determine whether the cancer harbors the mutations that the drug targets. Only a small fraction of lung cancer patients have *EGFR* gene mutations or *ALK* gene translocations, and only these patients will respond to the drugs. Treating lung cancer patients without these particular genetic alterations would be detrimental, as such patients would develop the toxic side effects of the drugs while their tumors progressed.

A second type of genome-based medicine focuses on the side effects and metabolism of the therapeutic agents, rather than the genetic alterations they target. At present, the dose of cancer drugs given to patients is based on the patients' size (body weight or surface area). But the therapeutic ratio of cancer drugs (ratio of the concentration that causes side effects to the concentration required to kill tumor cells) is generally low, particularly for conventional (nontargeted) therapeutic agents. Small changes in circulating concentrations of these drugs can make the difference between substantial tumor regression and intolerable side effects. Interrogation of the germline status of the genes encoding drug-metabolizing enzymes could substantially improve the outcomes of treatment by informing drug dosing (*127*). Optimally, this genome interrogation would be accompanied by pharmacokinetic measurements of drug concentrations in each patient. The additional cost of such analyses would be small compared with the exorbitant costs of new cancer therapies—for recently approved drugs, the cost is estimated to be $200,000 to $300,000 per quality life year produced (*128*).

Challenges

One challenge of genome-based medicine in oncology is already apparent from the opportunities described above: All of the clinically approved drugs that target the products of genetically altered genes are directed against kinases. One reason for this is that kinases are relatively easy to target with small molecules and have been extensively studied at the biochemical, structural, and physiologic levels (*129*). But another reason has far deeper ramifications. The vast majority of drugs on the market today, for cancer or other diseases, inhibit the actions of their protein targets. This inhibition occurs because the drugs interfere with the protein's enzymatic activity (such as the phosphorylation catalyzed by kinases) or with the binding of the protein to a small ligand (such as with G protein–coupled receptors). Only 31 of the oncogenes listed in tables S2 and S3 have enzymatic activities that are targetable in this manner. Many others participate in protein complexes, involving large interfaces and numerous weak interactions. Inhibiting the function of such proteins with small drugs is notoriously difficult because small compounds can only inhibit one of these interactions (*130*, *131*).

Though one can at least imagine the development of drugs that inhibit nonenzymatic protein functions, the second challenge evident from table S2 poses even greater difficulties: A large fraction of the Mut-driver genes encode tumor suppressors. Drugs generally interfere with protein function; they cannot, in general, replace the function of defective genes such as those resulting from mutations in tumor suppressor genes. Unfortunately, tumor suppressor gene–inactivating mutations predominate over oncogene-activating mutations in the most common solid tumors: Few individual tumors contain more than one oncogene mutation (Fig. 5).

The relatively small number of oncogene mutations in tumors is important in light of the intrametastatic heterogeneity described earlier. To circumvent the inevitable development of resistance to targeted therapies, it will likely be necessary to treat patients with two or more drugs. The probability that a single cancer cell within a large metastatic lesion will be resistant to two agents that target two independent pathways is exponentially less than the probability that the cell will be resistant to a single agent. However, if the cancer cell does not contain more than one targetable genetic alteration (i.e., an oncogene mutation), then this combination strategy is not feasible.

Given the paucity of oncogene alterations in common solid tumors and these principles, can targeted therapeutic approaches ever be expected to induce long-term remissions, even cures, rather than the short-term remissions now being achieved? The saviors are pathways; every tumor suppressor gene inactivation is expected to result in the activation of some growth-promoting signal downstream of the pathway. An example is provided by *PTEN* mutations: Inactivation of the tumor suppressor gene *PTEN* results in activation of the AKT kinase (Fig. 8). Similarly, inactivation of the tumor suppressor gene *CDKN2A* results in activation of kinases, such as cyclin-dependent kinase 4, that promote cell cycle traverse (*132*). Furthermore, inactivation of tumor suppressor gene *APC* results in constitutive activity of oncogenes such as *CTNNB1* and *CMYC* (*133*–*135*).

We believe that greater knowledge of these pathways and the ways in which they function is the most pressing need in basic cancer research. Successful research on this topic should allow the development of agents that target, albeit indirectly, defective tumor suppressor genes. Indeed, there are already examples of such indirect targeting. Inactivating mutations of the tumor suppressor genes *BRCA1* or *BRCA2* lead to activation of downstream pathways required to repair DNA damage in the absence of BRCA function. Thus, cancer cells with defects in *BRCA1* or *BRCA2* are more susceptible to DNA damaging agents or to drugs that inhibit enzymes that facilitate the repair of DNA damage such as PARP [poly(adenosine diphosphate–ribose) polymerase] (*136*). PARP inhibitors have shown

Box 2. Highlights

1. Most human cancers are caused by two to eight sequential alterations that develop over the course of 20 to 30 years.

2. Each of these alterations directly or indirectly increases the ratio of cell birth to cell death; that is, each alteration causes a selective growth advantage to the cell in which it resides.

3. The evidence to date suggests that there are ~140 genes whose intragenic mutations contribute to cancer (so-called Mut-driver genes). There are probably other genes (Epi-driver genes) that are altered by epigenetic mechanisms and cause a selective growth advantage, but the definitive identification of these genes has been challenging.

4. The known driver genes function through a dozen signaling pathways that regulate three core cellular processes: cell fate determination, cell survival, and genome maintenance.

5. Every individual tumor, even of the same histopathologic subtype as another tumor, is distinct with respect to its genetic alterations, but the pathways affected in different tumors are similar.

6. Genetic heterogeneity among the cells of an individual tumor always exists and can impact the response to therapeutics.

7. In the future, the most appropriate management plan for a patient with cancer will be informed by an assessment of the components of the patient's germline genome and the genome of his or her tumor.

8. The information from cancer genome studies can also be exploited to improve methods for prevention and early detection of cancer, which will be essential to reduce cancer morbidity and mortality.

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encouraging results in clinical trials when used in patients whose tumors have inactivating mutations of BRCA genes (*137*).

Further progress in this area will require more detailed information about the signaling pathways through which cancer genes function in human cancer cells, as well as in model organisms. One of the lessons of molecular biology over the past two decades is that pathway functions are different, depending on the organism, cell type, and precise genetic alterations in that cell (*138*). A pertinent example of this principle is provided by results of treatment with drugs inhibiting mutant BRAF kinase activity. In the majority of patients with melanomas harboring (V600E; V, Val; E, Glu) mutations in the *BRAF* gene, these drugs induce dramatic (though transient) remissions (*108*). But the same drugs have no therapeutic effect in colorectal cancer patients harboring the identical *BRAF* mutations (*139*). This observation has been attributed to the expression of EGFR, which occurs in some colorectal cancers but not in melanoma and is thought to circumvent the growth-inhibitory effects of the BRAF inhibitors. With this example in mind, no one should be surprised that a new drug that works well in an engineered tumor in mice fails in human trials; the organism is different, the cell type is usually different, and the precise genetic constitutions are always different. The converse of this statement—that a drug that fails in animal trials will not necessarily fail in human trials—has important practical consequences. In our view, if the biochemical and conceptual bases for a drug's actions are solid and the drug is shown to be safe in animals, then a human trial may be warranted, even if it does not shrink tumors in mice.

Genome-Based Medicines of the Future

Cancer genomes can also be exploited for the development of more effective immunotherapies. As noted above, typical solid tumors contain 30 to 70 mutations that alter the amino acid sequences of the proteins encoded by the affected genes. Each of these alterations is foreign to the immune system, as none have been encountered during embryonic or postnatal life. Therefore, these alterations, in principle, provide a "holy grail" for tumor immunology: truly tumor-specific antigens. These antigens could be incorporated into any of the numerous platforms that already exist for the immunotherapy of cancer. These include administration of vaccines containing the mutant peptide, viruses encoding the mutant peptides on their surfaces, dendritic cells presenting the mutated peptide, and antibodies or T cells with reactivity directed against the mutant peptides (*140*).

To realize these sorts of therapeutics, several conditions must be met. First, the mutant protein must be expressed. As cancer cells generally express about half of the proteins that are encoded by the human genome (*141*), this condition is not limiting. Second, as most proteins affected by mutations are intracellular, these mutations will not be visible to the immune system unless the mutant residue is presented in the context of a human leukocyte antigen (HLA) protein. Based on in silico analyses of binding affinities, it has been estimated that a typical breast or colorectal cancer contains 7 to 10 mutant proteins that can bind to an individual patient's HLA type (*142*). These theoretical predictions have recently gained experimental support. Studies of mouse tumors have identified mutant genes and shown that the corresponding peptides can induce antitumor immunity when administered as vaccines (*143*). Moreover, clinical trials of brain cancer patients immunized against a mutant peptide have yielded encouraging results (*144*).

As with all cancer therapies that are attractive in concept, obstacles abound in practice. If a tumor expresses a mutant protein that is recognizable as foreign, why has the host immune system not eradicated that tumor already? Indeed, immunoediting in cancers has been shown to exist, resulting in the down-regulation or absence of mutant epitopes that should have, and perhaps did, elicit an immune response during tumor development (*145*, *146*). Additionally, tumors can lose immunogenicity through a variety of genetic alterations, thereby precluding the presentation of epitopes that would otherwise be recognized as foreign (*147*). Though these theoretical limitations are disheartening, recent studies on immune regulation in humans portend cautious optimism (*148*, *149*).

Other Ways to Reduce Morbidity and Mortality Through Knowledge of Cancer Genomics

When we think about eradicating cancer, we generally think about curing advanced cases—those that cannot be cured by surgery alone because they have already metastasized. This is a curious way of thinking about this disease. When we think of cardiovascular or infectious diseases, we first consider ways to prevent them rather than drugs to cure their most advanced forms. Today, we are in no better position to cure polio or massive myocardial infarctions than we were a thousand years ago. But we can prevent these diseases entirely (vaccines), reduce incidence (dietary changes, statins), or mitigate severity (stents, thrombolytic agents) and thereby make a major impact on morbidity and mortality.

This focus on curing advanced cancers might have been reasonable 50 years ago, when the molecular pathogenesis of cancers was mysterious and when chemotherapeutic agents against advanced cancers were showing promise. But this mindset is no longer acceptable. We now know precisely what causes cancer: a sequential series of alterations in well-defined genes that alter the function of a limited number of pathways. Moreover, we know that this process takes decades to develop and that the incurable stage, metastasis, occurs only a few years before death. In other words, of the one million people that will die from cancer this year, the vast majority will die only because their cancers were not detected in the first 90% of the cancers' lifetimes, when they were amenable to the surgeons' scalpel.

This new knowledge of cancer (Box 2) has reinvigorated the search for cures for advanced cancers, but has not yet permeated other fields of applied cancer research. A common and limited set of driver genes and pathways is responsible for most common forms of cancer (table S2); these genes and pathways offer distinct potential for early diagnosis. The genes themselves, the proteins encoded by these genes, and the end products of their pathways are, in principle, detectable in many ways, including analyses of relevant body fluids, such as urine for genitourinary cancers, sputum for lung cancers, and stool for gastrointestinal cancers (*150*). Equally exciting are the possibilities afforded by molecular imaging, which not only indicate the presence of a cancer but also reveal its precise location and extent. Additionally, research into the relationship between particular environmental influences (diet and lifestyle) and the genetic alterations in cancer is sparse, despite its potential for preventative measures.

The reasons that society invests so much more in research on cures for advanced cancers than on prevention or early detection are complex. Economic issues play a part: New drugs are far more lucrative for industry than new tests, and large individual costs for treating patients with advanced disease have become acceptable, even in developing countries (*151*). From a technical standpoint, the development of new and improved methods for early detection and prevention will not be easy, but there is no reason to assume that it will be more difficult than the development of new therapies aimed at treating widely metastatic disease.

Our point is not that strenuous efforts to develop new therapies for advanced cancer patients should be abandoned. These will always be required, no matter our arsenal of early detection or preventative measures. Instead, we are suggesting that "plan A" should be prevention and early detection, and "plan B" (therapy for advanced cancers) should be necessary only when plan A fails. To make plan A viable, government and philanthropic organizations must dedicate a much greater fraction of their resources to this cause, with long-term considerations in mind. We believe that cancer deaths can be reduced by more than 75% in the coming decades (*152*), but that this reduction will only come about if greater efforts are made toward early detection and prevention.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/339/6127/1546/DC1
Tables S1 to S5

10.1126/science.1235122

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From: [redacted]]
Sent: Thursday, January 16, 2014 4:21 PM
To: Predith, Ashley
Subject: The Land rover Presidential Proposal http://landroverpresedentialproposal.blogspot.com/

Hello from Chuck Thompson Please view the Land Rover Presidential Proposal and contact me 6[redacted]

The Watchmen Program

#1 Will- Better protect the nation from Terrorist Attacks from the inside out
.#2 Will- Nationwide create millions of Jobs for Citizens within and Military personnel coming home.
#3 Will- Generate billions of New Revenue for the Federal & Local Government to reconcile their books

Video Presentation

http://landroverpresedentialproposal.blogspot.com

From: [redacted]]
Sent: Thursday, January 16, 2014 4:22 PM
To: Predith, Ashley
Subject: The Department of USDA & Energy Worldemergency Renewable Centers Proposal

Hello from Chuck Thompson Please click on Energy Centers Proposal link VIDEO https://www.youtube.com/watch?v=BbFfQW9-Bc8

My direct contact is [redacted]. I would like the meet with you to give the full details of this Emergency Energy Presentation. Thanks

The Department of USDA & Energy Centers Proposal

Building a Nationwide Green Zero- Grid Facilities Industry

To bring a nation on board adopting a New Green Facilities Industry building Homes, Schools Offices, Medical Units, and Portable Farms the Countries Citizens must see it. The People must be able to touch and feel this New Way of Life, this New Green Living Industry on display; within their world where they live, work and play.

The Watchmen Program will be a nationwide vehicle. The Department of Energy, other (federal & local) agencies, including organizations such as (WGES) World Green Energy Symposium, will place all the wonderful renewable ideals into action. The private sector working with government agencies placing this essential, renewable, change, front and center within

Energy City Centers where the country citizens live, is The Adoption Key

The Land Rover Presidential Proposal

Video Presentation http://www.youtube.com/watch?v=uWRkgK_VhyY

Complete details are spelled out in the publication titled "Reconciling Us or Revolution.
http://blip.tv/world-emergency-news/reconciling-us-or-revolution-5977014

The Proposal within is called The Watchmen Program.
http://www.youtube.com/watch?v=B-QT4MVa2ng&feature=youtu.be

Webinar Video link is Titled Worldemergencyradio State of the Union Address
http://wecomments.blogspot.com/2011/08/worldemergency-radio-state-of-union.html

"

From: [redacted]
Sent: Thursday, January 16, 2014 4:25 PM
To: Predith, Ashley
Subject: The Watchmen Program Security Answer To All Economical & Ecological Problems

Having trouble viewing this email? wewatchgov.com
Hi, just a reminder that you're receiving this email because you have expressed an interest in WorldEmergency. Don't forget to add [redacted] to your address book so we'll be sure to land in your inbox!

You may unsubscribe if you no longer wish to receive our emails.

#10 Thousand New Physical Health Care Exchange Centers Solves sign up troubles. Centers will Generate Two Trillion Dollars in New Revenue; paying all Affordable Health Care Cost. No Taxes Needed

Heath Exchange Centers will sign up 7 Million People before year ends. Help Centers will include the HHS, USDA, DOE Established by the Watchmen Program .

Hello from Chuck Thompson

Please View Video [redacted]

10 Thousand Renewable Energy Nationwide Information Offices for Health Care, & other Local, Federal Services

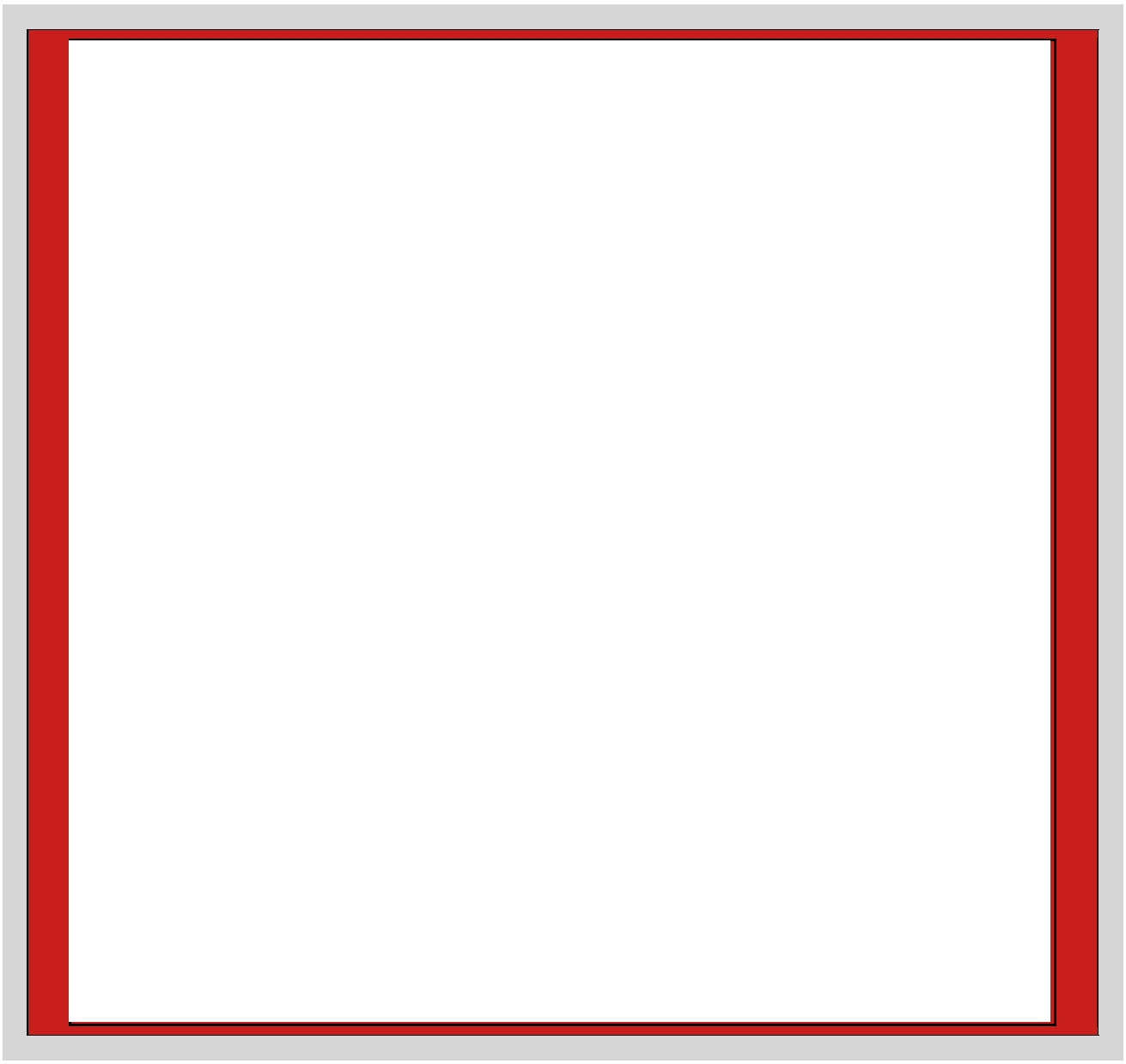
. My direct contact is [redacted]

.

I would like the meet with you to give the full details of this Renewable Energy Exchange Centers Presentation.

Building a Nationwide Green Zero- Grid Facilities Industry

Emergency Health Care Energy USDA Centers Video



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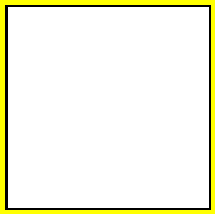
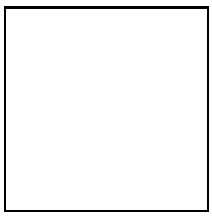
4 View The Watchmen Program Presentation

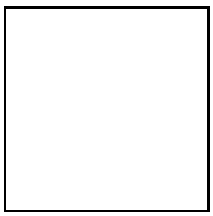
View Video Economic, Job, Health Care Act Presentaion

6  All Video Presention- Wewatchgov.com

#7  Ch uck Thompson R esume Link

http://weinf.blogspot.com/2011/12/chuck-thompson-resume.html





P238 fuel for ASRG

From: "David Czuba" [redacted]
Date: Fri, January 24, 2014 4:42 pm
To: pcast@ostp.gov

Hello PCAST,

I am writing to comment on NASA's budget and mission as a deep space exploration advocate. Without advanced propulsion methods, the U.S. Is limited in conducting deep space missions. The Advanced Stirling Radioisotope Generator (ASRG) and connecting program to produce Plutonium 238 fuel for powering the generator have been cut from NASA's budget. Deep space missions depend on this technology. For evidence, I point to the recent wake-up signal sent to the ESA's Rosetta spacecraft on its way to intercept a comet. Rosetta is powered by solar panels, but the spacecraft had to endure a 31 month sleep as it flew beyond 800 million kilometers, outside the Sun's ability to power the solar panels.

If the federal government has firmly decided to end the ASRG program, then private commercial space industry must be given the opportunity to pursue its further development. However, government would need to sanction private access to P238 fuel. This is extremely unlikely, whether produced internally or purchased from foreign interests, due to security. I strongly urge PCAST to recommend increasing the budget to re-institute our national ASRG and P238 programs. Let's reach for the stars! Thank you.

David Czuba
[redacted]