Cancer mutations and targeted therapies in cells, mice and patients

Alberto Bardelli

Institute for Cancer Research and Treatment
University of Torino - Medical School
Le analisi molecolari sui tumori umani, oggi possibili grazie al completamento del progetto genoma, stanno portando alla definizione di un nuovo paradigma:

"Il trattamento individualizzato del paziente oncologico sulla base del profilo molecolare delle sue cellule neoplastiche"
“Cancer is, in essence, a genetic disease. Although cancer is complex, and environmental and other nongenetic factors clearly play a role in many stages of the neoplastic process, the tremendous progress made in understanding tumorigenesis in large part is owing to the discovery of the genes, that when mutated, lead to cancer.”

Bert Vogelstein (1988)

*NEJM* 1988; 319:525-532.
Bert Vogelstein  
Ken Kinzler  
Victor Velculescu  

Johns Hopkins University Medical School  
The Howard Hughes Medical Institute
Argomenti

- Il cancro: una malattia dei geni
- Le cause del cancro
- I geni del cancro
- Le terapie personalizzate
IL CANCRO NEL 2009

27 milioni  Nuovi casi

17 milioni  Decessi

75 milioni  Pazienti
Il cancro: una malattia genetica

Prima mutazione
(può essere ereditata)

Seconda mutazione
Il cancro è una malattia dei geni
Il cancro........

AGAGTTCCTGCTCG
AGGGTTATGCACCG
CGTTAGGAAATCT
CGTTAGGAAATCT

AGAGTTCCTGCTCG
AGGGTTATGCACCG
CGTTAGGAAATCT
CGTTAGGAAATCT

TCCTTTGACGACTC
TCCTTAGAGGACCTC
PRINCIPALI CAUSE DELL’ INSORGENZA DEI TUMORI UMANI
Cosa danneggia i nostri geni?

Agenti chimici

Agenti fisici

Eredità

Virus

DNA
I NOSTRI GENI “INVECCHIANO”

Età

Incidenza tumori
La nostra vita media si allunga progressivamente
The population pyramid for China unfolds over a 100 (1950-2050) year period and the aging of the population becomes quite obvious.

For the period 1950 to 1995 the pyramid is based on population estimates of the UN Population Division; the data for 2000 to 2050 are from the most recent medium variant UN population projection.
CANCER WORLDWIDE BURDEN 2005

11 million New Cases
6.8 million Deaths
25 million Living with Cancer
CANCER □ WORLDWIDE BURDEN-2030

27 million New Cases

17 million Deaths

75 million Living with Cancer
Prevenzione
Prevenzione
Prevenzione
Prevenzione
Stomach Cancer - Women

Year

Standardized Mortality Ratio


Bulgaria

Czechoslovakia

Hungary

Poland

Romania
Argomenti

• Il cancro: una malattia dei geni
• Le cause del cancro
• I geni del cancro
• Le terapie personalizzate
Il cancro è una malattia dei geni
I geni del cancro
• THE HUMAN GENOME PROJECT
• THE CANCER GENOME PROJECT(s)
THE HUMAN GENOME PROJECTs

Initial sequencing and analysis of the human genome
Nature 409, 2001
The Genome International Consortium

The Sequence of the Human Genome
Science 2001
Celera
THE CANCER GENOME PROJECT

All cancers occur due to abnormalities in DNA sequence. Throughout life, the genome within cells of the human body is exposed to mutagens and suffers mistakes in replication. These errors influence cells in progressive, subtle divergence of the DNA sequence in each cell from that originally constituted in the fertilised egg. Occasionally, one of these somatic mutations alters the function of a critical gene, providing growth advantage to the cell in which it has occurred and resulting in the emergence of an expanded clone derived from this cell. Acquisition of additional mutations, and consequent waves of clonal expansion result in the evolution of the mutinous cells that invade surrounding tissues and metastasise. One in three people in the Western world develop cancer and one in five die of the disease. Cancer is therefore the commonest genetic disease.

The identification of genes that are mutated and hence drive oncogenesis has been a central aim of cancer research since the advent of recombinant DNA technology. The Cancer Genome Project is using the human genome to identify cancer genes in selected cancer types.

Data Resources
- Cancer Gene Census
- COSMIC: Catalogue Of Somatic Mutations In Cancer
- CGP Resequencing Studies
- CGP Cancer Cell Line Project
- CGP Copy Number Analysis in Cancer
Il genoma umano = Enciclopedia
Il genoma umano = 46 cromosomi
Il genoma umano = Enciclopedia

Courtesy of Bert Vogelstein
Il genoma umano

• Ogni gene è una singola pagina che contiene circa ~1500 caratteri

Courtesy of Bert Vogelstein
Il genoma umano

• Ogni gene è una pagina che contiene circa 1500 caratteri

• I geni sono organizzati in libri (cromosomi) di circa 1000 pagine ognuno

Courtesy of Bert Vogelstein
Il genoma umano

- Ogni gene è una pagina che contiene circa ~1500 caratteri
- I geni sono organizzati in libri (cromosomi) di circa 1000 pagine ognuno
- Il Genoma è una enciclopedia di 46 libri (23 libri da ogni genitore)
Il genoma tumorale = Enciclopedia

- 44 pagine con errori di ortografia
- 11 pagine mancanti
- 8 pagine duplicate
Il genoma tumorale = Enciclopedia
In realtà è più semplice di così

Solo una piccola parte delle mutazioni/errori di ortografia sono coinvolti nello sviluppo dei tumori
Colorectal cancer

>1 million cases/year about
50% with metastatic disease

212,000 deaths/year in Europe

www.future-health-2007.com
Leading Sites of New Cancer Cases and Deaths – 2006 Estimates

Estimated New Cases

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>Breast</td>
</tr>
<tr>
<td>234,460 (33%)</td>
<td>212,920 (31%)</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>Lung &amp; bronchus</td>
</tr>
<tr>
<td>92,700 (13%)</td>
<td>81,770 (12%)</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>Colon &amp; rectum</td>
</tr>
<tr>
<td>72,800 (10%)</td>
<td>75,810 (11%)</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>Uterine corpus</td>
</tr>
<tr>
<td>44,690 (6%)</td>
<td>41,200 (6%)</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>34,260 (5%)</td>
<td>28,190 (4%)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>Melanoma of the skin</td>
</tr>
<tr>
<td>30,680 (4%)</td>
<td>27,930 (4%)</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>Thyroid</td>
</tr>
<tr>
<td>24,650 (3%)</td>
<td>22,590 (3%)</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>Ovary</td>
</tr>
<tr>
<td>20,180 (3%)</td>
<td>20,180 (3%)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Urinary bladder</td>
</tr>
<tr>
<td>20,000 (3%)</td>
<td>16,730 (2%)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Pancreas</td>
</tr>
<tr>
<td>17,150 (2%)</td>
<td>16,580 (2%)</td>
</tr>
<tr>
<td>All sites</td>
<td>All sites</td>
</tr>
<tr>
<td>720,280 (100%)</td>
<td>679,510 (100%)</td>
</tr>
</tbody>
</table>

Estimated Deaths

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>Lung &amp; bronchus</td>
</tr>
<tr>
<td>90,330 (31%)</td>
<td>72,130 (26%)</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>Colon &amp; rectum</td>
</tr>
<tr>
<td>27,870 (10%)</td>
<td>27,300 (10%)</td>
</tr>
<tr>
<td>Prostate</td>
<td>Prostate</td>
</tr>
<tr>
<td>27,350 (9%)</td>
<td>27,350 (9%)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Pancreas</td>
</tr>
<tr>
<td>16,090 (6%)</td>
<td>16,210 (6%)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Leukemia</td>
</tr>
<tr>
<td>12,470 (4%)</td>
<td>15,310 (6%)</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>10,840 (4%)</td>
</tr>
<tr>
<td>10,730 (4%)</td>
<td>10,840 (4%)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>8,840 (3%)</td>
<td>8,840 (3%)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Esophagus</td>
</tr>
<tr>
<td>10,000 (3%)</td>
<td>10,000 (3%)</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>Kidney &amp; renal pelvis</td>
</tr>
<tr>
<td>8,130 (3%)</td>
<td>8,130 (3%)</td>
</tr>
<tr>
<td>All sites</td>
<td>All sites</td>
</tr>
<tr>
<td>291,270 (100%)</td>
<td>273,560 (100%)</td>
</tr>
</tbody>
</table>

*Excludes basal and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Note: Percentages may not total 100% due to rounding.

©2006, American Cancer Society, Inc., Surveillance Research
Colon cancer in Italy

35,000 cases/year

About 17,000 with metastases
Mutational landscapes of cancer genomes: 

Mountains and Hills

Bardelli et. al., Science: 300 (2003)

• Cancer gene *mountains* and EGFR targeted therapies in colorectal cancers

• Parallel clinical trials in cells, mice and patients
Cancer mutations and targeted therapies
Anti EGFR therapy and colorectal cancer

Cetuximab or Panitumumab

Adapted from Ciardiello F. and Tortora G. NEJM 2008;358:1160-74
Who will respond to EGFR targeted therapies?

Responders (15-20%)  Non-Responders

2004
Mutations in the EGFR signalling pathway predict response to antibodies targeting the EGFR

Moroni et al., Lancet Oncology 2005
Benvenuti et al., Cancer Research. 2007
Di Nicolantonio et al., J Clin Oncol. 2008
Sartore-Bianchi A et al., Cancer Res 2009
Siena, Di Nicolantonio and Bardelli JNCI 2009
Bardelli and Siena J Clin Oncol 2010
De Roock, Martini et al, Lancet Oncology 2010
Di Nicolantonio, Arena et al., JCI 2011
De Roock, Di Nicolantonio et al, JAMA 2011
Responder (15%)

PIK3CA mutated and/or PTEN loss (15-20%)

BRAF mutated (8%)

KRAS-NRAS mutated (35-45%)

KRAS/PIK3CA mutated

BRAF/PIK3CA mutated

20-25% ? (quadruple negative)

Bardelli and Siena, *J Clin Oncol* 2010
Not all mutations are created equal......
KRAS mutations

G12 G13 L19 Q61 K117 A146 R164 R173

- GTP binding
- Effector binding
- Switch I: Effector/GAP interaction
- Switch 2: GEF interaction

- Hotspot K-Ras mutations
- Novel K-Ras mutations
- K-Ras SNP

RAS (inactive)

Farnesyl Geranylgeranyl

RAS (active)

GTP binding

GDP

GEP

GDI

GAP

Effectors: RAF/MAPK/ERK PI3K/AKT
The role of individual KAS mutations in CRCs

<table>
<thead>
<tr>
<th></th>
<th>G12 (27.2%)</th>
<th>G13 (5.8%)</th>
<th>OTHER</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>11.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>7.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>2.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>0.4%</td>
<td></td>
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</tr>
<tr>
<td>D</td>
<td>5.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.18%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0.02%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Not all KRAS mutations are created equal
Colorectal cancers
Not all KRAS mutations are created equal

Pancreatic cancers
**KRAS mutations:**
clinical results from cetuximab treated mCRC

**Response rate:**
analysis of 8 studies available in PubMed or from ASCO

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moroni Lancet Oncol 2005</td>
<td>31</td>
</tr>
<tr>
<td>Li vre Clin Cancer Res 2006</td>
<td>30</td>
</tr>
<tr>
<td>Di Fiore Br J Cancer 2007</td>
<td>59</td>
</tr>
<tr>
<td>Frattini Br J Cancer 2007</td>
<td>27</td>
</tr>
<tr>
<td>Benvenuti Cancer Res 2007</td>
<td>48</td>
</tr>
<tr>
<td>Khambata-Ford J Clin Oncol 2007</td>
<td>80</td>
</tr>
<tr>
<td>De Roock ASCO Proc 2007</td>
<td>37</td>
</tr>
<tr>
<td>Finocchiaro ASCO Proc 2007</td>
<td>81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>wt</th>
<th>RAS Mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders (n=82)</td>
<td>93.0%</td>
<td>7.0%</td>
</tr>
<tr>
<td>Non-Responders (n=312)</td>
<td>56.1%</td>
<td>43.9%</td>
</tr>
</tbody>
</table>
Association of KRAS p.G13D Mutation With Outcome in Patients With Chemotherapy-Refractory Metastatic Colorectal Cancer Treated With Cetuximab

Conclusions In this analysis, use of cetuximab was associated with longer overall and progression-free survival among patients with chemotherapy-refractory colorectal cancer with p.G13D-mutated tumors than with other KRAS-mutated tumors. Evaluation of cetuximab therapy in these tumors in prospective randomized trials may be warranted.

1. indicate that patients with KRAS-mutated tumors (NCBI Entrez Gene 3845) do not benefit from the anti–epidermal growth factor receptor (EGFR) monoclonal antibodies cetuximab and panitumumab.1 These retrospective analyses were performed independently, and for each analysis, KRAS wild-type vs mutant were studied grouping codons 12 and 13 mutations together, without subgroup analysis. Health authorities in the United States and Europe have indicated that patients with KRAS codon 12– or KRAS codon 13–mutated tumors should not receive cetuximab or panitumumab.2-4

However, indications exist that not all KRAS mutations are equal in their biological characteristics. First, the pattern of KRAS mutations is tumor-type spe-
Molecular bases of G12V versus G13D mediated resistance to cetuximab in cellular and animal models
Experimental design

**Cellular model**

Gene targeting (Knock-in approach)

- **KRAS:** G12D, G12V, G12C, G12A, G12S, G12R, G13D
- **BRAF:** V600E, **PIK3CA:** E545K (exon 9), H1047R (exon 20)

Biochemical validation (pathway activation)

**Measure drug response**
A

AAV-KRas-12V

AAX-Neo-12V

ITR NotI
ITR
NotI
NotI
ITR

ITR
ITR

NotI
ITR
ITR
NotI
ITR

NotI
ITR
ITR
NotI
ITR

B

Homologous recombination

KRAS WT CRC cells

Knock-in G12V
(or G12D / G12C)

C

SW48 KRAS WT

SW48 KRAS G12V

SW48 KRAS G13D

G12V (G35>G/T)

G13D (G38>G/A)

SW48 KRAS WT

SW48 KRAS G12V

SW48 KRAS G13D
KRAS G12V or G13D and chemotherapy in cellular models
KRAS G12V and G13D and ceruximab in cellular models

De Roock et al JAMA 2010
Homologous recombination

KRAS WT CRC cells

Knock-in G12V (or G12D / G12C)

Knock-in G13D
Cetuximab delays growth of SW48 tumor xenografts
Cetuximab does not affect growth of G12V tumors, but inhibits the growth of G13D tumor xenografts.
• Cancer gene *mountains* and EGFR targeted therapies in colorectal cancers

• Parallel clinical trials in cells, mice and patients
Xenopatients: a preclinical platform
Xenopatients for pre-clinical trials

DNA, RNA and protein extraction, FFPE blocks stored by the pathologist

Liver Met implanted s.c. in NOD SCID mice

Patient undergoing liver metastasectomy of CRC

Using this approach 180 samples were successfully engrafted since Oct 2008

A. Bertotti & L. Trusolino, Molecular Oncology, IRCC
The doctor’s perspective

Genome Medical Futures

Royal Mail Stamp Issue 25 February 2003
The patient's perspective

"Here's my sequence..."
Molecular Genetics Lab:
Federica Di Nicolantonio
Sabrina Arena
Miriam Martini
Emily Crowley
Elisa Scala
Carlotta Cancelliere
Sebastijan Hobor
Davide Zecchin
Simona Lamba
Michela Buscarino
Livio Trusolino
Andrea Bertotti
Josep Tabernero
Sabine Tejpar
Salvatore Siena
Andrea Sartore Bianchi
Marcello Gambacorta
alberto.bardelli@ircc.it