# I biomarcatori preditivi: efficacia, metodologie e corretta applicazione clinica

**Dott. Massimo Gion**
**Centro Regionale Biomarcatori,**
**Azienda ULSS12 Venezia,**

# The classical approach

## Prognostic and predictive factors

# Prognostic factors

Prognostic factors predict the risk of relapse and death in the absence of systemic therapy

# Predictive factors

- Predictive factors are associated with relative sensitivity and/or resistance to specific therapeutic agents
- In the case of a pure predictive factor, patient outcomes in the absence of specific treatment are the same, regardless of whether the marker results are “positive” or “negative”

*Special Report*

Clinical Cancer Research

# AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report: Advancing the Use of Biomarkers in Cancer Drug Development

Samir N. Khleif[1], James H. Doroshow[1], and William N. Hait[2]; for the AACR-FDA-NCI Cancer Biomarkers Collaborative

*Clin Cancer Res; 16(13); 3299–318.* *©2010 AACR.*

# Biomarker definition

A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

# Biomarker classification

- **Diagnostic Biomarkers**
- **Predictive Biomarkers**
- **Metabolism Biomarkers**
- **Outcome Biomarkers**

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

# Biomarker classification

- Predictive Biomarkers
  - Predict patients likely to respond to a specific agent
  - Predict patients likely to have an adverse event to a specific agent

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

# Biomarker classification

- Outcome Biomarkers
  - Forecast response
  - Forecast progression/recurrence
  - Forecast resistance

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

## To Predict ?

- To state or make a declaration about in advance, esp on a reasoned basis

## To Forecast ?

- To predict or calculate in advance

*Collins Thesaurus of English*

# Biomarkers in relation to treatment

Given the integrated role of biomarkers at the cellular and molecular levels, a more extensive semantic interpretation of the term “predictive” seems more adequate to the potential clinical application of biomarkers

# Biomarkers in relation to treatment what can they “predict”?

## Will the tumor respond to a specific agent?

- Classical predictive markers (primary resistance)

## Is the tumor still responding to the treatment ?

- Markers for monitoring response/failure

## Why the tumor does not respond any more ?

- Markers related to acquired resistance

# Fundamental principles of personalized cancer therapy

- A significant genomic heterogeneity, implicating an estimated 384 cancer genes including many protein kinases,  exists among tumors, even those derived from the same tissue of origin
- These differences can play an important role in determining the likelihood of a clinical response to treatment with particular agents

(McDermott U and Settleman J, J Clin Oncol 2009)

# Classical predictive markers (primary resistance)

- Key role for molecular tumor pathology in the assessment of individual tumours
- Recent developments in high-throughput technologies for gene sequencing, comparative genomic hybridization and single nucleotide polymorphism array analysis, and bioinformatics have made it possible to interrogate the cancer genome rapidly and comprehensively

(McDermott U and Settleman J, J Clin Oncol 2009)

LA CARATTERIZZAZIONE MOLECOLARE DEI TUMORI POLMONARI

**Anton**

LA C MOLECOLARE DEI TUMORI COL

**Alber**

LA CARATTERI RI MAMMARI

**Anna Sapino**

LA CARATTERIZZAZIONE MOLECOLARE DEL PANCREAS

**Gianfranco Delle Fave**

**Classical predictive markers (primary resistance)**

**No room for circulating biomarkers**

STATO DELL'ARTE E PROSPETTIVE DELLA TARGET THERAPY NEI TUMORI POLMONARI

**Giorgio Scagliotti**

STATO DELL'ARTE E PROSPETTIVE DELLA TARGET THERAPY NEI TUMORI GASTROENTERICI

**Carlo Aprile**

STATO DELL'ARTE E PROSPETTIVE DELLA TARGET THERAPY NEI TUMORI [illegible]

**Pierfranco Conte**

STATO DELL'ARTE E PROSPETTIVE DELLA TARGET THERAPY NEL MELANOMA MALIGNO

**Enzo Galligioni**

**Classical predictive markers (primary resistance)**

**No room for circulating biomarkers**

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- Many therapeutic agents may yield growth inhibitory effects that are not detectable by conventional phase II clinical trial design, traditional radiological modalities, or current tumor response scoring systems (eg, RECIST)
- Tumor regression is increasingly recognized as an unreliable end point; a number of novel agents (such as sorafenib) cause tumor stasis or lead to necrosis with minimal viable tumor tissue but no overall change in tumor size

(McDermott U and Settleman J, J Clin Oncol 2009)

# Markers for monitoring response/failure (1)

- Classical circulating biomarkers have not been extensively explored
- However, their relationship with tumor bulk represents an inherent  theoretical limitation

(Gion M et al, 2010)

# Markers for monitoring response/failure (2)

Newer modalities such as fluorodeoxyglucose PET able to distinguish between viable and nonviable tumor masses makes them ideal imaging techniques for testing therapeutic agents

(McDermott U and Settleman J, J Clin Oncol 2009)

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- Despite some impressive clinical successes with several of the kinase targeted therapies that have been developed, clinical experience dictates that most, if not all, treatment-responsive patients eventually experience relapse as a result of acquired drug resistance

(Settleman J et al,  Curr Opin Genet Dev 18:73-79, 2008)

- Despite some impressive clinical successes with several of the kinase targeted therapies that have been developed, clinical experience dictates that **most, if not all, treatment-responsive patients eventually experience** relapse as a result of **acquired drug resistance**

(Settleman J et al, Curr Opin Genet Dev 18:73-79, 2008)

# Factors contributing to resistance (i.e EGFR family tyrosine kinase inhibitors)

- EGFR resistance mutations
- KRAS mutations
- MET amplification
- Other signaling pathways
  - VEGF
  - IGF-1
  - The process of epithelial-mesenchymal transformation
- ... ?? ...

(Kosaka T et al, Journal of Biomedicine and Biotechnology, 2011)
(Giaccone G and Wang Y, Cancer Treatment Reviews 2011)

# Acquired resistance
# Pitfalls

- Distinct mechanisms of resistance can arise within the same disease setting, during treatment with the same kinase inhibitor
- Multiple distinct resistance mechanisms arise within the same patient
- Distinct resistance mechanisms arise within the same patient over the time

(Giaccone G and Wang Y, Cancer Treatment Reviews 2011)

- Various resistance mechanisms can be managed with treatments appropriately matched with a second-generation therapy
- Careful treatment decisions demand knowledge of *genomic features for the effective management* of drug resistance

(Giaccone G and Wang Y, Cancer Treatment Reviews 2011)

# Acquired resistance Shortcoming

- The genomic analysis of relapsed tumor tissue requires rebiopsy after disease progression
- Rebiopsy of relapse may be unfeasible or invasive in most solid tumors

(Giaccone G and Wang Y, Cancer Treatment Reviews 2011)

# Circulating biomarkers

May circulating biomarkers have a role…

1. … for the detection of the occurrence of acquired resistance, or …
2. … for the identification of molecular mechanisms underlying acquired resistance ?

# Circulating biomarkers

- Circulating Tumor Cells
- Circulating biomarker molecules
- Carriers bound biomarkers

# Detection of Mutations in *EGFR* in Circulating Lung-Cancer Cells

*Maheswaran S, Sequist LV, Nagrath S, Ulkus L, Brannigan B, Collura CV, Inserra E, Diederichs S, Iafrate AJ,. Bell DW, Digumarthy S, Muzikansky A, Irimia D, Settleman J, Tompkins RG, Lynch TJ, Toner M, and DA Haber*

N Engl J Med 2008; 359: 366-77

- *EGFR* mutational analysis performed on DNA recovered from CTCs and compared with those from the original tumor-biopsy specimens
- CTC isolated from 27 patients with metastatic non–small-cell lung cancer
- The expected *EGFR* activating mutation found in CTCs from 11/12 patients (92%)
- T790M mutation detected in circulating tumor cells collected from patients with *EGFR* mutations who had received tyrosine kinase inhibitors

(Maheswaran S, et al, N Engl J Med 2008; 359: 366-77)

- EGFR mutations identified in lung CTCs were concordant with the mutations in the primary tumor in 12/13 cases
- During longitudinal follow up of these cases, continued therapy was associated with the emergence of a TKs resistance-associated EGFR mutation, coincident with the development of clinical drug resistance

(Maheswaran S, et al, N Engl J Med 2008; 359: 366-77)

- CTC analyses after prolonged therapy showed the acquisition of additional EGFR mutations that were below detection in the primary tumor biopsy
- Serial analysis of CTCs may allow real-time monitoring of molecular evolution among tumor cells during the use of targeted cancer therapy that applies selective pressures toward the development of drug resistance mechanisms

(Maheswaran S, et al, N Engl J Med 2008; 359: 366-77)

**Circulating tumor cells: approaches to isolation and characterization**

*Yu M, Stott S, Toner M, Maheswaran S and Haber DA*

J. Cell Biol. 2011; 192(3): 373–382

# The biological background

To date, only limited information is available about:

- the numbers of CTC in the blood stream at different stages of cancer and in different types of cancer
- their molecular and biological heterogeneity
- their significance in the natural history of the disease

(Yu M, et al J. Cell Biol. 2011; 192: 373–382)

# The methodological background

- the development of appropriate, high throughput, and reliable technological platforms for rare tumor cell detection within blood specimens remains the critical impediment.
- In the absence of any gold standard with which to measure various technologies, defining their absolute accuracy, sensitivity, and specificity in detecting CTCs remains a challenge.

(Yu M, et al J. Cell Biol. 2011; 192: 373–382)

| **Assay System/ Platform** | **Enrichment** | **Detection** |
|---|---|---|
| CellSearch® | EpCAM-ab coupled ferrofluid | CK+, CD45- |
| EPISPOT | Depletion of CD45+ cells | Secreted proteins (CK19; MUC-1) |
| CTC-chip | EpCAM-ab coupled µ-posts | CK+, CD45- |
| MAINTRAC® | RBCs lysis | EpCAM+, CD45- |
| Ikoniscope® | Ficoll or filtration | EpCAM+, CK7/8+,<br>cr 7/8 (FISH) |
| Ariol® | RBCs lysis<br>CK and EpCAM-ab coupled µ-beads | CK8/18/19+, CD45- |
| RT-PCR methods | Immunomagnetic/gradient | mRNA for CK19/HER2/EpCA |

# Circulating biomarkers

- Circulating Tumor Cells
- Circulating biomarker molecules
- Carriers bound biomarkers

# Circulating biomarker molecules

## Innovative thecnologies

- ...omics
- Multiplexing
- …..

## Novel biomarker families

- Mechanisms related biomarkers
- Biomarkers associated to host immune response
- Nucleic acids related biomarkers
- …

# Mechanism related biomarkers

- **Cytokines/GF**
  - VEGF
  - IL8
  - IL6
  - IL10
  - IL18
  - TGFb1
  - HGF/SF & c-Met
  - ….
- **Markers of genetic imbalance**
  - erbB2
  - erbB1
  - erbB1R
  - ….
- **Adhesion molecules**
  - e-cadherine
  - ICAM-1
  - Laminin-5
  - Osteopontin
  - …
- **Proteinases**
  - MMPs
  - uPA system
  - SCCA
  - …..

# Mechanisms related biomarkers

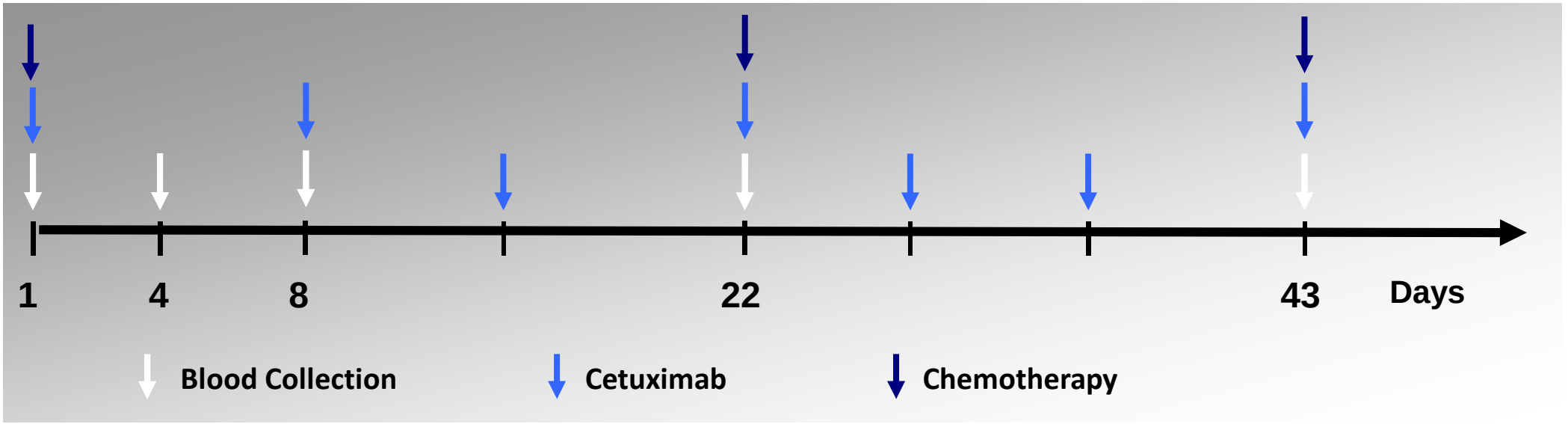
- They are based on an *a priori* biological rationale
- They are related to well known biological mechanism (signaling, angiogenesis, apoptosis , protease activation, …)
- They are associated to variations of tumor phenotype that are related to biological and/or clinical pattern (aggressiveness, responsiveness to given agents, ...)

**Phase II study of panitumumab, oxaliplatin, 5-FU, and concurrent radiotherapy as preoperative treatment in high-risk locally advanced rectal cancer patients (StarPan/STAR-02 Study)**

*Pinto C, Di Fabio F, Maiello E, Pini S, Latiano T, Aschele C, Garufi C, Bochicchio A, Rosati G, Aprile G, Giaquinta S, Torri V, Bardelli A, Gion M, Martoni A.*

Ann Oncol. 2011; 11: 2424-30

# Overview of Study design and Treatment Schema

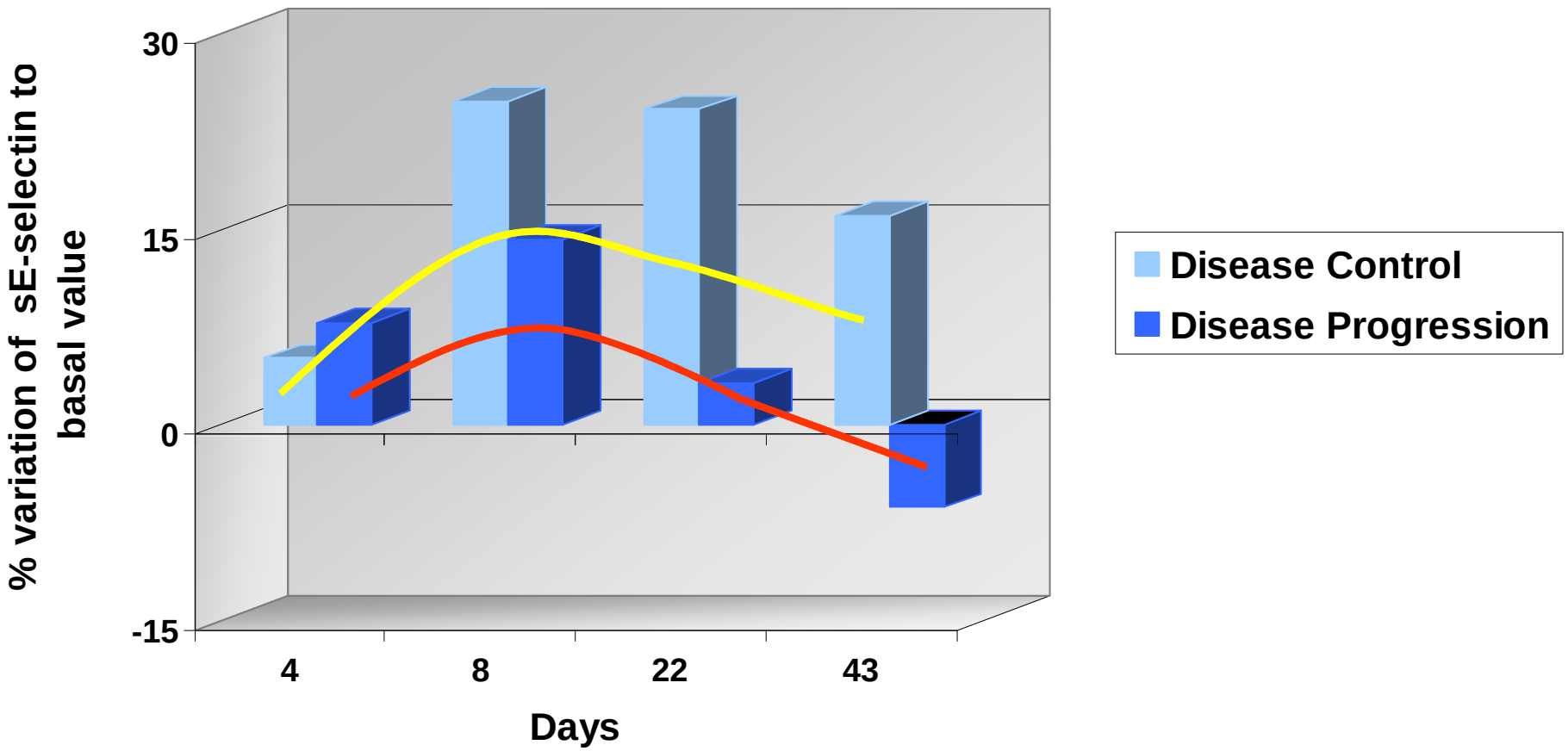



**BLOOD COLLECTION (days 1, 4, 8, 22, 43)**

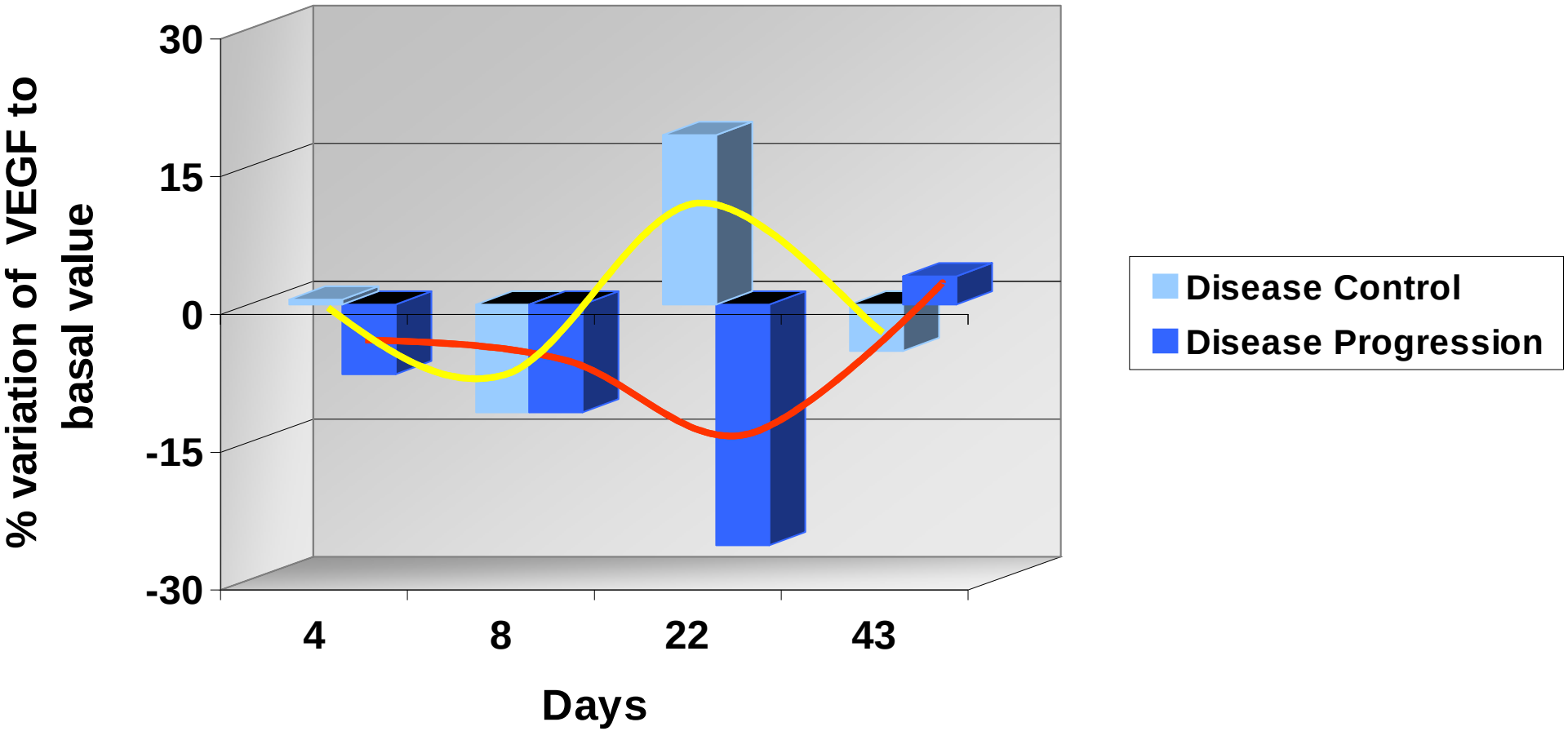
**CETUXIMAB ADMINISTRATION (weekly)**

**CHEMOTHERAPY (every 3 weeks)**

(Pinto et al., Annals of Oncology 2011)




(Pinto et al., Annals of Oncology 2011)




(Pinto et al., Annals of Oncology 2011)

# Surrogate markers for antiangiogenic therapy

- Rising levels of circulating factors (e.g. VEGF and placenta growth factor) were observed in response to antiangiogenic drugs or chemotherapy, possibly reflecting treatment-induced tumor hypoxia
- The practical utility of using drug induced increases in circulating factors as surrogate biomarkers remains to be demonstrated, and their use might be confounded by increases associated with tumor resistance or escape

Bocci G *et al*., *Cancer Res* 2004; **64:** 6616–6625
Willett CG *et al*. *J Clin Oncol* 2005 ; **23:** 8136–8139

**Biomarkers of angiogenesis for the development of antiangiogenic therapies in oncology: tools or decorations?**

*Sessa C, Guibal A, Del Conte GL and Rüegg C*

Nature Clinical Practice ONCOLOGY 2008; 5: 378-391

- Many biomarkers of angiogenesis have been proposed and investigated, but none has yet been validated for routine clinical use
- Many basic questions related to the assessment of tumor angiogenesis and monitoring antiangiogenesis therapies have remained unanswered
- In spite of the initial success n the field, biomarkers of angiogenesis are desperately required

(Sessa C, et al, Nature Clinical Practice ONCOLOGY 2008)

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(Sessa C, et al, Nature Clinical Practice ONCOLOGY 2008)

# Where is the problem?

- Biomarkers are currently measured in serum or plasma
- The blood recirculates thousand times/day and biomarkers in serum are cleared and processed thousand times each day mainly by liver and kidney
- Thus, the circulating levels of biomarkers produced and released by tumor tissue are expected to be continuously lowered by physiological metabolic pathways, thus remaining below the detection levels

# Where is the problem?

- Serum or plasma is a material intrinsically not suited for the detection of small amounts of biomarker(s), expecially if they have a fast  with a rapid half life
- This is true for both protein and nucleic acid biomarkers, specially those RNA related

# Circulating biomarkers

- Circulating Tumor Cells
- Circulating biomarker molecules
- Carriers bound biomarkers

# Body fluid derived exosomes as a novel template for clinical diagnostics

*Keller S, Ridinger J, Rupp AK, Janssen JWG and Altevogt P*

Journal of Translational Medicine 2011, 9: 86

- Exosomes contain proteins, miRNAs and mRNAs (exosome shuttle RNA, esRNA) that could serve as novel platform for diagnosis
- Exosomes from body fluids carry esRNAs which can be analyzed and offers access to the transcriptome of the host organism
- The exosomal lipid bilayer protects the genetic information from degradation

(Keller S, et al, Journal of Translational Medicine 2011, 9: 86)

**Characterization of soluble and exosomal forms of the EGFR released from pancreatic cancer cells**

*Adamczyk KA, Klein-Scory S, Tehrani MM, Warnken U, Schmiegel W, Schnölzer M, Schwarte-Waldhoff I*

Life Sci 2011 Aug 29; 89(9-10): 304-12

Pancreatic cancer cells secrete:

- a 110 kDa soluble form of the EGFR (sEGFR) representing the ligand binding extracellular EGFR domains and presumably released by ectodomain shedding
- an exosomal full-length intact EGFR (170 kDa)
- an exosomal 65 kDa processed form that corresponds to the intracellular kinase domain

(Adamczyk KA, et al, Life Sci 2011)

- The detailed characterization of diverse EGFR forms released by pancreatic cancer cells in vitro and presumably in vivo bears important implications for functional studies, for the validation of soluble EGFR as a serum biomarker and for the design of targeted therapies

(Adamczyk KA, et al, Life Sci 2011)

**Survivin is released from cancer cells via exosomes**

*Khan S, Jutzy JMS, Aspe JR, McGregor DW, Neidigh JW, and Wall NR*

Apoptosis. 2011; 16(1): 1–12

- Survivin has been implicated in apoptosis inhibition and the regulation of mitosis in cancer cells
- In the present study, we describe for the first time the exosome release of Survivin to the extracellular space both basally and after proton irradiation-induced stress

(Khan S, et, Apoptosis. 2011; 16: 1–12)

**Exosomes as biomarker treasure chests for prostate cancer**

*Duijvesz D, Luider T, Bangma CH, Jenster G*

Eur Urol 2011 May; 59(5): 823-31

- Exosomes represent their tissue origin
- Purification of prostate- and PCa-derived exosomes will allow us to profile exosomes, providing a promising source of protein and RNA biomarkers for PCa
- This profiling will contribute to the discovery of novel markers for the early diagnosis and reliable prognosis of PCa
- Although the initial results are promising, further investigations are required to assess the clinical value of these exosomes in PCa

(Duijvesz D, et al, Eur Urol 2011 59: 823-31)

# Che fare?

Preparasi a valutare correttamente nuovi marcatori:

1. di famiglie diverse
2. con tecnologie diverse
3. con criteri decisionali diversi (cinetici)
4. in matrici diverse

# Come fare?

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# Critical areas to advance the use of biomarkers in cancer drug development

1. Biospecimens (3 recommendations)
2. Analytic Performance (3 recommendations)
3. Standardization and Harmonization (4 rec.)
4. Bioinformatics (2 rec.)
5. Collaboration and Data Sharing (3 rec.)
6. Regulatory Issues (6 rec.)
7. Stakeholder Education and Communication (3 rec)
8. Science Policy (3 rec.)

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

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(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

# Regulatory Issues

- Develop best practices on **codevelopment of therapeutics and diagnostics**
- Develop best practices for biomarker assays based on a composite of **multiple** individual **biomarkers**
- Develop best practices for **retrospective-prospective** study designs for clinical qualification of biomarkers
- Develop best practices on **adaptive clinical trial** designs for using biomarkers in drug development
- Develop best practices on **alternative prospective** trial designs for companion diagnostics

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

## … retrospective-prospective design, … adaptive clinical trial, … alternative prospective trial design??

- The ultimate goal is to develop study architectures which can allow for a reliable application and evaluation of biomarkers that were not anticipated at the beginning of a trial but could have been identified at the end of the trial

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

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# Biospecimens

- Establish quality standards and promote routine quality assessment of biospecimens acquired for research
- Develop a publicly available national oncology resource of biospecimen reference standards for biospecimen quality assessment and analytic validation
- Promote an infrastructure and climate supportive of biospecimen research

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

# Biospecimens

- The generation of large collections of high-quality biospecimens necessary for biomarker research and development may require significant changes to the current infrastructure and research climate

(AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report, Clin Cancer Res 2010)

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