



1° Convegno di Immunometria Sud Italia **NUOVI BIOMARCATORI ONCOLOGICI**



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VIII° Seminario di Oncologia Medica

“I Markers Tumoriali”

Bari, 29 Gennaio 1988

- Anticorpi monoclonali marcati nella diagnosi e nella terapia delle neoplasie: problemi attuali e prospettive future (G. L. Buraggi)
- Utilità dei modelli sperimentali nella interpretazione clinica dei marcatori tumorali circolanti ([E. Bombardieri](#))
- Utilità limiti e sviluppi futuri degli anticorpi monoclonali nella pratica clinica (M. I. Colnaghi)
- Ruolo dei markers tumorali nei linfomi maligni. Aspetti generali e classificazione ([M. Correale](#))
- Ruolo dei markers tumorali nei linfomi maligni. Correlazioni clinico-prognostiche ([E. Maiello](#))
- Labeling index, recettori ormonali ed ormonosensibilità nel carcinoma mammario ([A. Paradiso](#))
- Marcatori tumorali nel citosol del carcinoma mammario (M. Gion)
ecc. ecc.

Allo stato attuale, per varie motivazioni, vi è rinnovato interesse per i BIOMARCATORI ONCOLOGICI in generale e, soprattutto, un crescente ottimismo circa l'identificazione di nuovi marker.



Contestualmente, alcune importanti limitazioni impongono di procedere con cautela e dopo le opportune validazioni



Definizione di Biomarcatori Oncologici

Tumor markers (TM) are surrogate indicators that increase or decrease the clinician's suspicion that future clinically important events, such as cancer onset, recurrence, or progression or patient death, will or will not happen, and/or that a specific treatment will decrease the risk of such events.

(NACB Document Clin. Chem. (2008) 54: 8 e1- e10)

Mentre i TM “classici” si propongono essenzialmente per il monitoraggio delle neoplasie, oggi si cercano nuovi TM orientati sempre più anche verso gli aspetti diagnostici e le indicazioni terapeutiche.

DIAGNOSI-----TERAPIA

ESTIMATED NEW CASES and DEATHS by SEX, US, 2008

Estimated New Cases				Estimated Deaths		
	Both Sexes	Male	Female	Both Sexes	Male	Female
All Sites	1,437,180	745,180	692,000	565,650	294,120	271,530

(American Cancer Society: Cancer Facts & Figures 2008)

Early detection of various forms of cancer before they spread and become incurable is an important incentive for physician and research scientists.

One of the best ways to diagnose cancer early, aid in its staging and prognosis, predict or monitor therapeutic response, is to use serum or tissue biomarkers.

DRUGS IN DEVELOPMENT

TOP 10 TOPICS

(R & D Directions 2008; 14 (9): 20)

1. 667 for cancer
2. 252 for CNS disorders
3. 206 for cardiovascular disorders
4. 186 for infections
5. 138 for diabetes
6. 109 for pain/inflammation
7. 92 for respiratory disorders
8. 85 for blood disorders
9. 71 for gastrointestinal disorders
10. 54 for dermatological disorders

FARMACI BIOLOGICI / TERAPIA PERSONALIZZATA

BIOMARKERS IN CANCER DRUG DEVELOPMENT

- Pharmacodynamic Biomarkers
- Predictive Biomarkers
- Outcome Biomarkers or Surrogate Endpoints

(Glassman RH, Ratain MJ Clin Pharmacol Ther 2009; 85(2): 134-5)

CANCER BIOMARKERS

Nowadays,

CANCER BIOMARKERS can be:

- DNA
- RNA
- **PROTEINS**
- METABOLITES
- CIRCULATING CELLS
- PROCESSES, such as apoptosis, angiogenesis, signal trasduction or proliferation.

Table 3 Human biological fluids: a source for biomarker discovery.

Human biological fluid	Cancer type
Plasma	Broad spectrum of diseases
Serum	Broad spectrum of diseases
Cerebrospinal fluid	Brain
Nipple aspirate fluid	Breast
Breast cyst fluid	Breast
Ductal lavage	Breast
Cervicovaginal fluid	Cervical and endometrial
Stool	Colorectal
Pleural effusion	Lung
Bronchoalveolar lavage	Lung
Saliva	Oral
Ascites fluid	Ovarian
Pancreatic juice	Pancreatic
Seminal plasma	Prostate and testicular
Urine	Urological

da Nature Clin Pract Oncol 2008, 5: 588-99

TUMOR TISSUE INTERSTITIAL FLUID

Every Era of **Biomarkers Discovery** seems to be associated closely with the emergence of a new and powerful **Analytical Technology**



IMMUNOASSAY DEVELOPMENT

- **1959 First radio-immunoassay for insulin**
- Immunoassays
 - Competitive
 - Homogeneous
 - Non-Competitive
 - Heterogeneous
 - Isotopic
 - Non-Isotopic (1976 First Chemiluminescent)
- **1975 Monoclonal Antibodies** (Hybridomas)
- Automated Immunoassay Analyzers
(Special Sections)
- Automated ImmunoChemistry Analyzers
(Robotic Track, Integrated Platforms)
- **1991 Antibody microarrays (Ekins)**
1996 First genomic microarrays (Affymetrix)

The Future of Immunoassay Technology

It appears to be headed in two main directions:

1. Continued improvement in immunodetection methods for very high-sensitivity applications
2. Multiplex analysis for the simultaneous measurement testing of many analytes

Dynamic Range of Proteins in Plasma

At the high abundance end: **Albumin** (35-50 mg/mL)



At the low abundance end: **IL6** (0-5 pg/mL)

It is clear that sub-pg/mL sensitivities will be needed to allow measurement of many of the tissue proteins that might leak into the plasma.

[The theoretical limit is 10^{-7} pg/mL (~ 1 zeptomolar)]

(Anderson NL, Anderson NG Mol Cell Proteomics 2002; 1: 845-67)

MULTIPLEX ANALYSIS

The maturation of NANOTECHNOLOGY (associated to BIOINFORMATIC) has enabled the development of MICROARRAYS for multivariate immunoassay detection.

Parallel analyses (opposed to serial analyses) provide opportunities to distinguish patterns, signatures, portraits.

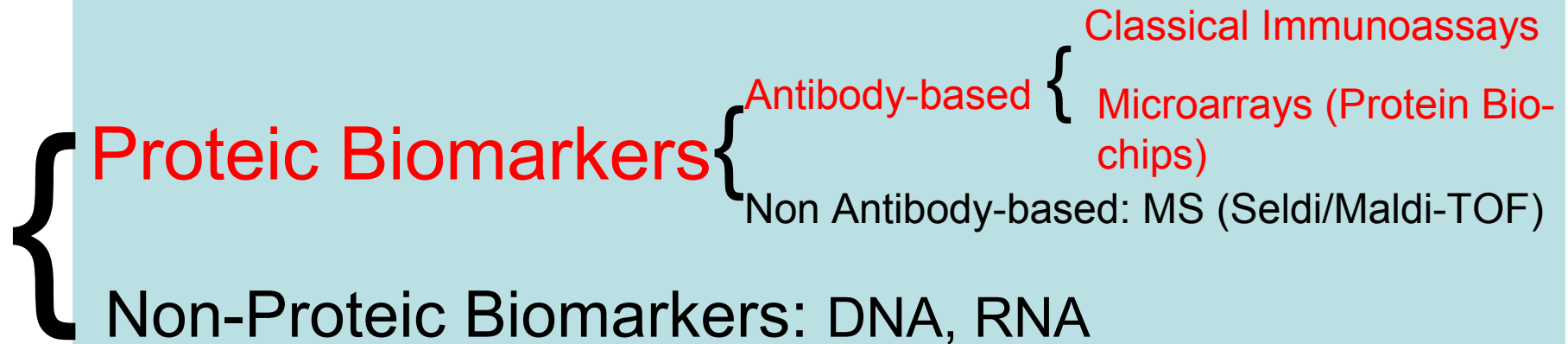
Single analyte, panel (3-10), profiles (>20).

The Cancer Secretome: a reservoir of biomarkers

There is a growing consensus that no single cancer biomarker is sensitive and specific enough... A feasible strategy ... is to measure a combination of proteomic biomarkers.

Xue H et al. J Translational Medicine 2008,6: 52

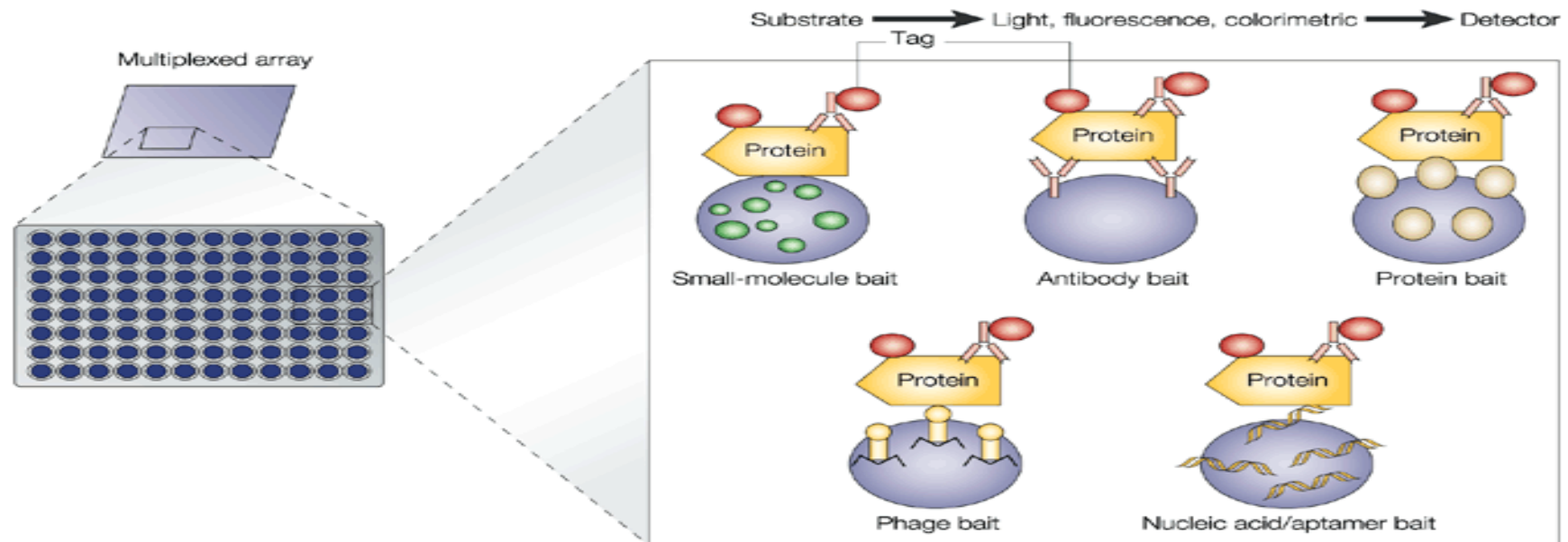
TABLE OF CANCER BIOMARKERS



More recent high-throughput **PROTEOMIC METHODS:**

- Protein Microarrays
- MS (Seldi/Maldi-TOF)

Protein microarrays consist of an array of protein samples, or protein baits, immobilized on a solid phase. The array is queried with a mixture of labelled proteins containing analytes of interest. The analyte proteins are captured and can be detected using fluorescence, colorimetric or chemiluminescence means.



Nature Reviews | Drug Discovery

[Clinical proteomics: translating benchside promise into bedside reality](#)

Emanuel F. Petricoin, Kathryn C. Zoon, Elise C. Kohn, J. Carl Barrett & Lance A. Liotta

Nature Reviews Drug Discovery 1, 683-695 (September 2002)

Evidence Investigator

The versatile analyser for immunossays and DNA testing (però per i TM)



BIOCHIP ARRAY TECHNOLOGY

PRINCIPLES OF MICROARRAYS

(NACB TUMOR MARKER GUIDELINES 2008, mod.)

ADVANTAGES :

- Simoultaneous access to many tests
- Less reagent
- Less sample volume
- Shorter incubation time
- Minimal reagent wastage
- High sensitivity and quantification (?)

PRINCIPLES OF MICROARRAYS

(NACB TUMOR MARKER GUIDELINES 2008, mod.)

LIMITATIONS :

- They are still evolving and need clinical validation (i.e. panel)
- Difficulties for standardization and reproducibility
- Discrepancies with other technologies
- Artifacts related to several variables
- QC systems must be established

All these breakthroughs by modern technologies have paved the way for countless new avenues for biomarker identification

However, very few serum TM have been introduced to the clinic over the past 15 (and also 5) years and

despite the optimism, ASCO and NACB do not encourage the widespread use of TM unless they affect patient outcome measures.

Ma a questo punto che si fa?



Compiti attuali dell'Immunometria nel campo dei TM

- Uso appropriato
- Standardizzazione
- Automazione
- Approfondimento “Vecchi” TM
- Studio Nuovi TM

NACB QUALITY REQUIREMENTS FOR THE USE OF TUMOR MARKERS

(Sturgeon CM, Diamandis EP NACB Laboratory Medicine Practice Guidelines 2009)

- **Pre-analytical requirements** - choice of tumor marker, specimen type, specimen timing, sample handling.
- **Analytical requirements** – assay standardization, internal and external quality control, interferences.
- **Post-analytical requirements** – reference intervals, interpretation and reporting of tumor marker results.

Vecchi e Nuovi BIOMARCATORI ONCOLOGICI

Ci sono già molti TM che meritano di essere studiati e valutati con giusti criteri nelle sedi idonee:

- Isoforme e Precursori del PSA
- Callicreine
- Pro Gastrin-Releasing Peptide (Pro-GRP)
- Soluble Mesothelin Related Proteins (SMRP) - Mesomark
- Glypican-3
- Isoforme dell' S-100 (A4, A8, A9,...), **ecc.ecc.**

HE4

(Human Epididymal secretory protein 4)

Questa glicoproteina, overespressa nel ca. ovarico, costituisce un esempio del nuovo approccio per identificare i TM:

- cDNA microarrays: 101 trascritti;
- RT-PCR: 12 trascritti, di cui 2 (HE4 e MSLN) i + significativi;
- Quantificazione dell'HE4 nel siero;
- Kit EIA manuale;
- Immunodosaggio automatizzato.

HE4 + CA-125

l'efficacia della combinazione

Stime di sensibilità – validazione crociata

Analisi di esclusione	Sensibilità per Ca ovarico vs. patologia benigna a:		
Marcatore/i	Specificità 90%	Specificità 95%	Specificità 98%

CA 125	61,2%	43,3%	22,9%
HE4	77,6%	72,9%	64,2%
CA 125 + HE4	80,7%	76,4%	71,6%
CA 125+HE4+CA 72-4	82,1%	78,8%	71,5%

HE4 + CA-125

l'efficacia della combinazione

Stime di sensibilità e validazione crociata

RG. Moore et al. Gynecol. Oncol. 2008; 108: 402-8

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- Come singolo marcatore, HE4 è più sensibile del CA-125
- La combinazione di HE4 e CA-125 è più sensibile di ciascuno dei marcatori da solo
- **Nello stadio I, HE4 da solo era il marcatore più sensibile**

Indice predittivo per Ca ovarico

- Per la discriminazione di una massa pelvica può essere utilizzato un indice predittivo, calcolato in base ai risultati di ARCHITECT CA-125II e di HE4 EIA.
- Se l'indice è elevato, la paziente dovrebbe essere valutata da uno specialista in oncologia ginecologica
(RG Moore et al. Gynecol. Oncol. 2009; 112: 40-6)

Donne in premenopausa $PI = -12,0 + 2,38 \times LN[HE4] + 0,0626 \times LN[CA125]$

Donne in postmenopausa: $PI = -8,09 + 1,04 \times LN[HE4] + 0,732 \times LN[CA125]$

La predittività per Ca ovarico viene calcolata come rapporto percentuale tra il valore esponenziale di PI ed il suo reciproco:

$$\text{Probabilità predittiva \%} = v.e.(PI) / [1 + v.e. (PI)] * 100$$

Humoral Immunity directed against Tumor-Associated Antigens as potential Biomarkers

Over the past decade, it has been demonstrated that cancer is immunogenic, and multiple tumor antigens have been identified in cancer patients.

Humoral immunity, or the development of autoantibodies against tumor antigens, may be used as a marker for cancer exposure.

However, antibodies have limited sensitivities as single analytes...mentre un pannello di anticorpi...

Lu H et al. J Proteome Res 2008; 7:1388-94

Anderson KS et al. J Proteome Res 2008; 7: 1490-9

IMMUNOPROTEOMICS

Circulating Serum Antibodies reflect information from all around the human body, by providing a molecular imprint of antigens that are related to autoimmune diseases, cancer or infection.

(Tjalsma Proteomics Clin Appl. 2008; 2: 167-180)

They have many appealing features, which make them attractive biomarkers.

IMMUNOPROTEOMICS

- The amplification cascade governed by the humoral immune system causes a **surplus of circulating antibodies** after the appearance of the cognate (low abundance) antigen in the human body;
- Although an antigen may be present only briefly, the corresponding immuneresponse is likely to be **persistent**;
- The **half-life** of antibodies varies between 7 and 20 days, long enough to minimise daily fluctuations;
- Antibodies are **highly stable** compared to many other serum proteins.

**ANCORA GRAZIE A TUTTI PER
LA PARTECIPAZIONE**

