Development of personalized medicine in oncology

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Target therapy in oncology

- **Targeted cancer therapies:** drugs that block cancer growth by interfering with specific molecules (targets) involved in cancer growth and progression

- **Target:** a protein specifically or preferentially expressed in cancer vs. normal cells

- Targeted drugs are generally safer and better tolerated

- Clinical practice is evolving from the choice of the drug treatment based on symptoms and immunohistochemistry classification of the tumor versus the **personalized medicine**

- **Personalized medicine:** characterization of the molecular background of the single tumor (patient) to prevent, diagnose, and select the more appropriate treatment
Protein kinases are a family of enzymes involved in key cellular functions, often deregulated in cancer.

518 protein kinases have been identified in the human genome, characterized by a conserved catalytic domain.

Alterations at the DNA level or at gene expression levels drive kinase oncogenicity.

Patients harboring tumors driven by these gene rearrangements are excellent candidates for targeted kinase inhibitor therapies.
Imatinib (Gleevec): the first approved kinase inhibitor in CML

- A chromosomal translocation between chr9 and chr22 allows the formation of the Philadelphia chromosome, with an aberrant fusion protein: BCR-ABL
- BCR-ABL expression drives the proliferation of CML cells
- Imatinib treatment stops BCR-ABL signaling and causes CML cells apoptosis
- Imatinib was approved by FDA in 2001 for the treatment of Chronic Myeloid Leukemia

Pray, L. Nature Education 2008
CML patient survival probability in the last 30 years

- **Imatinib** (Gleevec) treatment has improved the 10-year survival rates from less than 20% in 1983 to around 92% in 2013, with a life expectancy near to the one of healthy individuals.

- 2\textsuperscript{nd}-generation inhibitors **nilotinib**, **dasatinib** (both approved in 2010), **bosutinib** (approved in 2012) and the 3\textsuperscript{rd}-generation inhibitor **ponatinib** (accelerated approval in 2012, approval in 2016), active on the T315I resistant-mutated kinase form, have been developed.

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Tariq, Haematologica 2016
Lung cancer is one of the Biggest Killers
A growing list of genetic alteration have helped in defining lung cancer subgroups
Drugs targeting these specific mutations have been developed and demostrated improved responses in clinical trials

Tsao AS., Scientific Advances in Lung Cancer 2015
### Treatment of NSCLC harboring EGFR mutations

- EGFR mutations (10–15% of NSCLC) lead to kinase over-expression and consequent unregulated cell growth and proliferation
- **Erlotinib, Afatinib** and **Gefitinib** inhibit mutated EGFR and are effective in NSCLC treatment
- **Osimertinib** is a third generation EGFR inhibitor active towards EGFR mutants (also T790M resistant mutation). It was recently approved by FDA for the first-line treatment of metastatic NSCLC patients with EGFR mutated

#### 56-year-old non-smoker metastatic NSCLC

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<tr>
<th>February 2013, Pre Erlotinib treatment</th>
<th>June 2013, On Erlotinib treatment</th>
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<td>Erlotinib treatment</td>
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<th>CT Head December 2015, Pre Osimertinib treatment</th>
<th>CT Head October 2016, On Osimertinib treatment</th>
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<td>Osimertinib treatment upon relapse</td>
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Bartholomew C., Respir Med Case Rep. 2017
Drug discovery workflow

**Identification of active leads**
Identify molecules that are active on the target protein.

**Optimisation phase**
Optimise the molecules' properties so that they are safe and effective.

**Preclinical development**
Preclinical trials in order to document that the compound is safe for entry into human trials.

**Confirmed phase III trials**
Pivotal trials to confirm efficacy and safety in larger patient populations.

**Identification and validation of target proteins**
Identify and validate the biological mechanism behind a disease.

**Identification of hit compounds**
Identify classes of molecules that have the potential to become pharmaceuticals.

**Candidate drugs**
Selection of substances with optimised properties for further development.

**Phase I and phase II**
The first clinical trial is carried out on healthy volunteers in order to document pharmaceutical safety, followed by patient trials in order to define the safe and effective dose.

**Registration and market launch**

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Genomics and bioinformatics support
The development of targeted therapies starts with the identification of the **target:**

- **GENE MUTATIONS:** BRAF V600E mutation in melanomas → BRAFmut inhibitor vemurafenib
- **GENE OVEREXPRESSION:** HER2 amplification in breast cancer → HER2 inhibitor trastuzumab
- **GENE FUSIONS:** BCR-ABL fusion protein → ABL inhibitor imatinib

It is also fundamental to select the right patient population, through the use of **predictive biomarkers**, which indicate the likelihood of response to a specific antitumor therapy:

- In some instances **biomarker** and **target** correspond
The search for novel rearrangements involving kinases may lead to the identification of new candidate targets for drug discovery.

Need to query large datasets to identify rare events, about 1-3% of tumoral samples.

Kinase overexpression can be used as read-out of the presence of a gene fusion event.

We have implemented KAOS (Kinase Automatic Outliers Search), a computational method for the automatic identification of genes selectively over-expressed in a very small fraction of samples (outliers).

Nuzzo A, Carapezza G, Di Bella S, Pulvirenti A, Isacchi A and Bosotti R
KAOS: a new automated computational method for the identification of overexpressed genes
BMC Bioinformatics 2016
KAOS validation on a public dataset

- The tool was validated on a selection of about 500 kinases from Cancer Cell Line Encyclopedia (CCLE) microarray data (917 human cancer cell lines from 24 different tissue types)

- The method identified several known and novel overexpressed kinases in specific cell lines

- NTRK1 (Neurotrophic Tyrosine Kinase Receptor 1) is expressed at high level in KM12 colorectal cancer cell line only
KM12 harbors a TPM3-NTRK1 rearrangement

- NTRK1 is a tyrosine kinase **NOT** expressed in colon

- NTRK1 anomalous overexpression is caused by a fusion of its C-terminal kinase catalytic domain with the oligomerization N-terminal domain of TPM3 (tropomyosin3), an ubiquitously expressed protein

- We had experimentally demonstrated that, upon rearrangement, the NTRK1 kinase domain is expressed as a fusion protein with TPM3 in KM12 cell lines

Ardini E, Bosotti R, Lombardi Borgia A et al. The TPM3-NTRK1 rearrangement is a recurring event in colorectal carcinoma and is associated with tumor sensitivity to TRKA kinase inhibition. Molecular Oncology 2014
Identification of a CRC tumor sample with TPM3-NTRK1 rearrangement

- We screened a panel of CRC surgical FFPE samples to estimate the frequency of NTRK1 rearrangements in CRC
- We used a RT-qPCR method to identify relative expression levels of mRNA encoding the intracellular and extracellular domains of NTRK1
- Out of 66 analyzed surgical samples, we identified one sample (T27) expressing detectable levels of mRNA encoding the intracellular but not the extracellular domain of NTRK1
- T27 was the only sample displaying strong anti-TRKA immunoreactivity
- We confirmed the TPM3-NTRK1 rearrangement by direct sequencing

![Graph showing expression levels of TPM3-NTRK1](image1)

![Image of tissue samples](image2)
- The NMS chemical collection includes a dedicated Purine Targeted Library (PTL) of ca. 70,000 compounds, suitable ligands for the kinase ATP pocket.
- The collection was tested on a panel of 39 cell lines and we observed a striking correlation between antiproliferative activity on KM12 cells and high biochemical potency against TRKA.

KM12 cell line is sensitive to the treatment with NTRK1 inhibitors.

**IC\textsubscript{50}: 19 nM**

\[
\frac{1}{IC_{50}} = 53 \text{ mM}^{-1}
\]
Entrectinib

- Entrectinib is a novel, potent orally available inhibitor of ALK, ROS1 and TRK family kinases

- It is exquisitely potent in vitro against cell lines dependent on the drug’s pharmacological targets and highly efficacious by oral administration and at well tolerated doses in TRKA, ROS1 and ALK-driven xenograft models

- Granted by the FDA the orphan drug designation for the treatment of Trks, ROS1 or ALK-positive NSCLC, orphan drug designation and rare pediatric disease designation for the treatment of neuroblastoma and for treatment of NTRK fusion-positive solid tumors

- Received by the FDA the break-through therapy designation in 2017

- Entrectinib clinical development was initiated by NMS and continued by Ignyta (San Diego, CA, USA), through two phase I studies and a Phase II potentially registrative study which is almost completed

- Ignyta was recently acquired by Roche, who will complete entrectinib development up to commercialization

Patient population selection: the Archer FusionPlex strategy

- In vitro and/or in silico technologies have been extensively applied to the identification of rearranged kinases and their partner genes.
- Recently, more sensitive approaches have been developed based on Next Generation Sequencing (NGS).
- Anchored Multiplex PCR (Archer FusionPlex) is an NGS-based system that allows the identification of rearrangements involving selected kinases of interest, such as ROS1, ALK, NTRK1.
- Library preparation is based on a PCR amplification using two primers: a universal primer and a primer designed on the target gene, to allow the detection of gene fusion regions.
- The library is then subjected to NGS sequencing and allows the identification of the rearrangement partner.
Analysis of a TRKA positive tumor sample

- 61-year-old patient diagnosed with colon adenocarcinoma. The patient progressed early on two standard treatment lines.

- Endoscopic biopsy of the tumor mass showed strong positivity for TRKA protein by IHC analysis, with clear cytoplasmic distribution.

- FISH analysis showed the absence of the red centromeric signal from the break-apart probe in 68% of analyzed nuclei.

- These analysis suggested the presence of a rearrangement of NTRK1 gene.

*Milione M et al.,* Identification and characterization of a novel SCYL3-NTRK1 rearrangement in a colorectal cancer patient *Oncotarget 2017*
Identification of a novel SCYL3-NTRK1 by Archer

- Applying Archer NGS system a new NTRK1 rearrangement was identified.

- The rearrangement results from an inversion within chromosome 1, fusing exons 1–11 of the SCY1 Like Pseudokinase 3 (SCYL3) gene with exons 12–17 of NTRK1 gene.

- We transfected IL3-dependent Ba/F3 cells with SCYL3-NTRK1 cDNA construct. As consequence of the expression of the fusion protein, Ba/F3 cells acquired IL3-independent proliferation capability, demonstrating that SCYL3-NTRK1 is an oncogenic driver.
Analysis of an Alk positive tumor sample

- A 53-year-old patient diagnosed with metastatic colon adenocarcinoma in disease progression after standard therapies

- High levels of ALK protein observed by IHC, suggesting the presence of a rearrangement of ALK gene, also consistent with FISH analysis

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*Amatu et al., Novel CAD-ALK gene rearrangement is drugable by entrectinib in colorectal cancer Br J Cancer.2015*
Using Archer NGS assay we identified a new ALK gene fusion.

The rearrangement results from an inversion within chromosome 2, fusing exons 1–35 of the carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD) gene with exons 20–29 of ALK (C35-A20).

The gene fusion was confirmed by PCR/Sanger sequencing.
Upon these findings the patient was enrolled in the ALKA-372-001 phase I study of entrectinib, showing a response 4 weeks after the beginning of treatment, with a decrease in the sum of the target lesions by 38%.

**TAC before treatment**
showing the presence of liver metastasis

**TAC after 1 month of treatment**
clearly shows a reduction of liver metastasis
For further information, please visit www.nervianoms.com