for the development of specific inhibitory therapy for which in essence 3 approaches can be conceived;
– Antisense oligonucleotides or ribozymes that can serve to inhibit RNA transcription and receptor synthesis;
– Specific monoclonal antibodies targeting either receptor or ligand;
– Competitive inhibition at the phosphorylation binding domains of the intracellular site of the receptor once ligand-receptor interaction has occurred;

To date, monoclonal antibodies targeting either HGF/SF and C-Met and small molecule C-Met tyrosine kinase inhibitors have been developed and are currently undergoing clinical phase I and II studies, meanwhile showing preliminary hints of clinical activity in papillary renal cell cancer and gastric cancer. Currently, both C-Met selective and broad spectrum receptor TKI are in the clinic, and bearing in mind the experience and knowledge obtained in the last two decades with the use of this type of targeted or cancer cell specific therapy, it is important to asses whether selective C-Met inhibition or more broad spectrum receptor (tyrosine kinase) inhibitory activity is to be preferred. It is obvious that this will partly depend on the toxicities observed, as well on our ability to demonstrate proof-of-principle pharmacodynamic activity. It will also be crucial to assess whether single agent therapy or combinations with other systemic treament options (cytotoxic chemotherapy, other targeted agents, radiotherapy) will result in optimal antitumor activity, and therefore it can be foreseen that, based upon results obtained in preclinical models, in the near future a plethora of (randomised) phase II and III studies incorporating C-met inhibiting agents will be performed.

5 INVITED

Biological roles of PI 3-kinase isoforms

B. Vanhaesebroeck[1]. [1]*Institute of Cancer, Centre for Cell Signalling, London, United Kingdom*

The PI 3-kinase signalling axis is one of the most frequently mutated signalling pathways in cancer. Interference with this pathway is therefore considered an attractive therapeutic approach in oncology. The exact signalling connections in the PI3K pathway are still being unraveled, and it is presently not clear at what level it is best to interfere, i.e. upstream, downstream or at the level of PI3K itself, or combinations. In addition, mammals have 8 distinct isoforms of PI3K, and global inhibition of all isoforms of PI3K may have substantial toxicity in vivo. Therapeutic intervention at the level of PI3K itself may therefore have to be centered on specific (subsets of) PI3K isoforms. This might be exemplified by haematological malignancies where the often the p110delta isoform of PI3K is predominant. It has been particularly difficult to gain insight into the physiological roles of PI3K isoforms by classical mouse gene targeting/knock-out approaches. We have pioneered the use of so-called 'kinase knockin' mice in which we have created germline inactivating mutations in the ATP-binding site of PI3K isoforms. This strategy more faithfully mimics pharmacological inhibitors than the classical knock-out approaches, and has allowed us to uncover isoform-selective roles of several isoforms of PI3K. These genetic strategies, together with pharmacological approaches using newly developed small molecule inhibitors offer a powerful platform to unravel the roles of PI3K isoforms in the normal organism and in disease. An overview of these efforts will be presented.

6 INVITED

Notch as a potential therapeutic target in cancer

L. Miele[1], P. Rizzo[1], C. Osipo[1], K. Foreman[1], M. Bocchetta[2], D. Tonetti[3]. [1]*Loyola University Chicago Medical Center, Breast Cancer Program, Cardinal Bernardin Cancer Center, Illinois, USA;* [2]*Loyola University Chicago Medical Center, Thoracic Oncology Program, Cardinal Bernardin Cancer Center, Illinois, USA;* [3]*University of Illinois, Chicago, USA*

Background: There is increasing evidence for a key oncogenic role of the Notch signaling pathway in several solid tumors. The best characterized to date is breast cancer, with accumulating data on lung cancer. Notch signaling regulates proliferation, survival and differentiation of cancer cells, as well as communication between tumor and endothelial cells. Additionally, there is considerable evidence that Notch is among the pathways that control the self-renewal of "cancer stem cells". Consequently, a number of Notch inhibitors are being developed. Among these, γ-secretase inhibitors (GSIs), are in early clinical trials. However, Notch signaling is notoriously context- and dose-dependent, and is regulated by a complex network of cross-talk interactions with other pathways, some of which include potential therapeutic targets. Understanding these interactions will make it possible to design rational combination regimens.
Materials and Methods: We used several in vitro and in vivo cancer models, including ERα+ (T47D and MCF-7), ERα-, HER2Neu+ (SKBr3), ERα-, HER2/Neulow (MDA-MB-231) breast cancer cells, A549 lung adenocarcinoma cells and others, as well as clinical specimens.
Results: We discovered that: (1) In ERα+ breast cancer cells, estrogen suppresses Notch signaling by regulating the cellular distribution of Notch-1 and causing membrane accumulation of inactive Notch-1. Either estrogen withdrawal, mimicking the effects of aromatase inhibitors, or SERMS such as tamoxifen, cause re-activation of Notch signaling and increased dependence on Notch for proliferation, survival and invasion. Combinations including a GSI and an anti-estrogen are synergistically effective in vitro and in vivo. Such combinations are now being tested in the clinic; (2) In at least one model of tamoxifen resistance, Notch-4 plays a key role and GSIs are highly effective in vitro and in vivo, reversing tamoxifen resistance; (3) ERα-, PR-, Her2low MDA-MB231 cells are highly sensitive to Notch inhibition in vitro and in vivo; (4) Her2/Neu overexpression inhibits Notch signaling by modulating membrane availability of Notch ligands. Treatment of Her2/Neu overexpressing breast cancer cells with trastuzumab or with a tyrosine kinase inhibitor (TKI) causes re-activation of Notch signaling. Combination regimens including a Her2/Neu targeting agent and a GSI are at least additive in vitro and decrease tumor recurrence in vivo. Trastuzumab resistant cells are highly sensitive to GSIs, which reverse trastuzumab resistance; (5) Mammosphere formation by "breast cancer stem cells" is dramatically inhibited by GSIs; (6) In lung adenocarcinoma (ACL) cells, hypoxia stabilizes Notch-1 via HIF-1α. Hypoxic ACL cells are exquisitely dependent on Notch for survival and highly sensitive to GSIs.
Conclusions: Our data support the therapeutic investigation of Notch inhibitors in combination with: (1) endocrine therapy in ERα-positive breast cancers and in some tamoxifen-resistant breast cancers; (2) Her2/Neu targeted agents in Her2/Neu overexpressing and trastuzumab-resistant breast cancers; (3) anti-angiogenic agents or other hypoxia inducers in ACL. Additional combinations for rational targeting of triple-negative breast cancer cells and "cancer stem cells" are currently being studied.