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# NEWSLETTER MARCH - APRIL

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# SPOTLIGHT


## Professor Marc Vooijs

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MARC VOOIJS

## BIOGRAPHY

Marc Vooijs is a professor at the department of radiotherapy/ Maastro Lab at the MUMC+, closely affiliated with the out-patient Maastro Clinic, the largest radiotherapy clinic in the southeast of The Netherlands. Maastro Lab has a state-of-the-art preclinical mouse cancer model pipeline with different cancer models (spontaneous, orthotopic, primary patient xenografts) and imaging modalities (BLI, PET, MRI) and a small animal irradiator and CT. There are 25 people working in the lab with four principle investigator-led teams consisting of six postdocs, ten PhDs and five technicians.

Marc undertook his PhD in the Netherlands Cancer Institute with Anton Berns and conducted postdoctoral fellowships at Washington University in Saint Louis with Raphael Kopan and in Hubrecht Institute with Hans Clevers. In 2006 he started his own group at the department of pathology (head: Paul van Diest) at the UMC-Utrecht. In 2010 he moved to the MUMC to become head of laboratory research at Maastro.

His research is focussed on mechanistic insight into signal transduction by NOTCH family proteins and their context-dependent role in cancer development and treatment response, with an emphasis on tumour microenvironment and hypoxia. Furthermore, he is developing new reagents to monitor the NOTCH activation cascade under normal and pathophysiological conditions.

He has been awarded over three million Euros by an European Research Council (ERC) starting grant (ERC-Stg, NOTCH PROTEOLYSIS, 2008), ERC Proof of Concept (ERC, PoC, 2012 CAPNOTCH) and an ERC Consolidator grant (ERC-CoG, DIRECT, 2013). He has also received funding from the Dutch Cancer Society (KWF/NKB) and the Association for International Cancer Research (AICR 2013). In 2012 he was elected a member of the Young Academia Europe (YAE). He has been a fellow of the Dutch Cancer Society and has been awarded the prestigious van Nieuwenhoven award for biology from the Radboud University and in 2013 the Maastro research award. He holds one patent. Marc is co-organiser of the first international symposium on small animal imaging and precision radiotherapy in 2013 in Maastricht.

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## WHY NOTCH YET?

### *Unmet need*

Resistance to radiotherapy is a common cause of treatment failure and tumour recurrence. The effect of radiotherapy is largely restricted by toxicity of late responding tissues. Drugs that either enhance tumour cell sensitivity or prevent normal tissue toxicity will enable dose de-intensification on normal tissues while achieving effective tumour cell kill. Whereas there have been successful randomised phase III trials reporting radiosensitisation and increased local control in squamous head and neck cancer using antibodies targeting the EGFR receptor (cetuximab) or by targeting tumour hypoxia by reoxygenation (carbogen, nicotinamide) or hypoxic cytotoxins (nimorazole), effects on overall survival are still limited.

At present, it is unclear whether these tumour-cell specific approaches really target the most resistant, malignant and recurrent tumour cells. Increasing evidence indicates that a small subpopulation of malignant cells in tumours with properties of stem cells also referred to as cancer stem cells (CSC) or tumour-initiating cells are drivers of intra-tumour heterogeneity that underlies poor treatment responses and tumour recurrence. For example, in preclinical models for glioblastoma CD133+ (a CSC marker) cells have a high tumour-initiating capacity and are more radio-resistant compared to the bulk of CD133- glioma cells.

Similar treatment-resistant CSC have been identified in lung, breast and prostate cancer and many studies have shown that CSC are also resistant to chemotherapies (e.g. docetaxel, cisplatin). Studies in human colorectal cancer have shown that the stem cell gene expression signature (from embryonic stem cells or intestinal stem cells) is associated with high-grade tumours and relapse. Taken together CSC may be a good target worth further investigating. How to target these cells? The NOTCH signalling pathway may be an attractive route, albeit not without hurdles.

### *NOTCH in cancer*

NOTCH signalling is a cell-to-cell communication system where adjacent ligand expressing and receptor-expressing cells interact. Ligand binding to NOTCH receptors leads to cleavage in the transmembrane domain of NOTCH by the intramembrane protease complex g-secretase. This causes the release of the membrane tethered cytoplasmic domain which translocates to the nucleus where it is recruited to the chromatin of targeted genes by the DNA binding RBP/CSL and Mastermind like proteins (MAML) resulting in activation of target gene transcription.

Most well described target genes are basic helix loop helix proteins of the HES and HEY family. The human genome encodes for four Notch receptors and up to five canonical ligands of the JAGGED and DELTA family, which are expressed in unique and overlapping patterns and perform essential functions during development and in adult tissues. Notch proteins function is highly context dependent. In certain tissues Notch proteins promote stem cell renewal (e.g. intestine), while in other tissues they promote differentiation (e.g. epidermis). Consequently both gain and loss of function of NOTCH proteins has been associated with cancer development, progression and poor outcome.

The best understood are mutations found in T cell acute lymphocytic leukaemia (T-ALL) where 60% of tumours harbour activating mutations in NOTCH1. Exome sequencing of human non small cell lung adenocarcinomas report activating mutations in NOTCH1 (10%) or loss of NUMB (30%), a negative regulator of the NOTCH pathway. In contrast a high frequency of loss of function of NOTCH1 (and to a lesser extent in NOTCH2 and 3) has been reported in ▼

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squamous carcinomas of the skin, lung and head and neck. Head and neck cancers with over-expression of wild type receptors, NOTCH ligands and target genes have also been identified. After TRP53, NOTCH1 is the most frequently deregulated pathway in these cancers. Mutations in NOTCH4 have not been reported in human tumours thus far.

Furthermore, NOTCH signalling is also a key factor in the tumour microenvironment. The NOTCH ligand DLL4 and vascular endothelial growth factor (VEGF) coordinate the sprouting and proliferation of tumour vessels in a coordinated fashion and inhibition of either VEGF or DLL4 functionally impairs vessel growth in tumours, causing tumour regression in preclinical xenograft models. In hypoxic cancer cells, induction of VEGF leads to DLL4 expression, which is a negative regulator of VEGF and blocks sprouting and branching of tumour vessels. Interestingly, anti-DLL4 treatment can suppress tumour growth in anti-VEGF resistant tumours (e.g. bevacizumab).

### *NOTCH and treatment resistance*

Recent evidence obtained from preclinical models implicates NOTCH signalling in the resistance to radiotherapy and chemotherapy providing new opportunities for therapeutic application. Initially in brain but now also in lung, breast and prostate cancer there is convincing evidence that NOTCH signalling is important in treatment-resistant and recurrent tumour cells reminiscent of CSCs (CD133+ or CD24low/-CD44high (e.g. in breast). Blocking NOTCH signalling in preclinical tumour models reduces tumour growth and sensitivity to radiation treatment while over-expression of NOTCH accelerates growth and promotes resistance to radiotherapy. Taken together these results in preclinical models suggest that NOTCH could potentiate the effect of radiotherapy at least in some tumours.

While there is still much to be learned on the mechanism by which NOTCH proteins confer treatment resistance, available results suggest that NOTCH signalling may increase the survival of hypoxic cells and of CSC. Because normal stem cells have been found in hypoxic niches, this provides a testable hypothesis regarding a hypoxic treatment resistant CSC niche in tumours that depend on NOTCH for survival.

### *NOTCH inhibitors in clinical trials*

Many preclinical studies have provided rationale for the use of NOTCH inhibitors in patients. Inhibitors of the g-secretase intramembrane protease complex (GSI) are potent inhibitors of NOTCH signalling *in vivo* (www.clinicaltriasl.gov). Currently there are around 40 registered phase I-II trials in many different tumour types. The first phase I trials using GSIs in T-ALL patients failed due to dose-limiting gastrointestinal (GI) toxicity. New intermittent dosing schemes of GSI in phase I studies seem to negate the initial observed GI toxicity related to on-target NOTCH inhibition in the gut stem cells that causes precocious secretory differentiation and severe diarrhoea. Using these new dosing schemes, much higher maximum tolerated doses are achieved and in one trial stable disease in ten out of 21 patients with high grade gliomas for more than four months was observed. Similarly, studies in patients with advanced breast cancer yield sustained stable disease. Although these results are encouraging and demonstrate potent anti-tumour activity of NOTCH inhibitors, some caution is warranted.

### *How to move forward*

While some preclinical studies have studied the effect of GSI in combination with chemotherapy, most have studied the efficacy of GSI as monotherapy. Because patients often receive combined treatments (chemo- and/or radiotherapy) or have relapsed with treatment resistant tumours,

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the intervention points for treatment are not comparable/known and treatments involving GSIs may therefore not be effective. This could lead to the dismissal of this potentially potent anti-cancer drug because of lack of understanding of the underlying biology similar to the failure of metalloprotease inhibitors in cancer therapy. Indeed, there seems to be a decline in the number of new clinical studies using GSIs, partly because we still lack the appropriate diagnostic, predictive and theranostic biomarkers for patient stratification and therapy monitoring.

### *What we need next*

Thus, while these first findings of NOTCH inhibitors in patients seem promising, more insight is needed to understand their full potential and shortcomings. Such insight will have to come from preclinical and basic research.

Most research is focussed on the role of NOTCH1. However, the role of other NOTCH proteins in cancer is increasingly being appreciated. Since these may have importance as distinct aspects of tumourigenesis (initiation, invasion, metastasis, drug resistance etc) or even opposing functions (oncogene versus tumour suppressor), the simple measurement of a receptor expression or a few target genes (e.g. HES, HEY) in current clinical studies as a readout for NOTCH activity is clearly insufficient. This is crucial because currently used NOTCH inhibitors are pan-NOTCH inhibitors, i.e. they inhibit processing through all NOTCH receptors and therefore may also inactivate its potential tumour suppressive functions. Therefore, robust reagents such as isoform specific antibodies, which measure cleaved and activated forms of NOTCH receptors, or NOTCH pathway-specific gene-signatures are needed to assess which receptor is active and how this affects outcome or treatment response. Once available, these reagents could be used as diagnostic reagents for patient stratification and as companion diagnostics during GSI treatment.

Secondly, because NOTCH signalling plays crucial roles in the tumour microenvironment, NOTCH inhibitors have the potential to concurrently kill tumour cells and block angiogenesis. This dual function may be exploited to provide a therapeutic window sensitive to NOTCH inactivation. A wrong timing however e.g. NOTCH inhibition of angiogenesis prior or during radiotherapy might increase tumour hypoxia and induce treatment resistance.

Thirdly, because most patients receive combination treatments, such combinations have to be tested first in preclinical models since the underlying biology will teach us how to most optimally schedule NOTCH inhibitors in combination with chemotherapy and/or radiation therapy. In addition, using high throughput screens in cell based systems, synthetic lethal combinations between NOTCH inhibitors and chemo- and radiotherapy may be found that can be directly tested in preclinical models.

Fourthly, many preclinical studies rely on the use of xenografts with human immortalised cancer cell lines in immune deficient recipients. It is evident that at least some aspects of tumour growth and treatment response differs (1) between cell lines and direct transplantation of human tumours (patient derived xenografts or PDX), (2) between tumours grown subcutaneously versus orthotopic models and (3) between immune-competent and immune-deficient models. Further, spontaneous tumour models in mice (GEMM) which mimic specific oncogenic mutations (e.g. KRas/p53 mutant) can produce with short latency and with high incidence tumours that resemble their human counterpart (e.g. NSCLC). While these murine cancer models are spontaneous and therefore most closely resemble human tumour development, there are also substantial differences in the genetics of mouse

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versus man that potentially impact on the extrapolation of these models into a clinical relevant setting. Moreover, in some models only (high numbers of) low-grade tumours develop and do not reflect the clinical presentation where most often only one primary tumour is detected at an advanced stage. Choosing the right model may, therefore, not be so straightforward.

### *In conclusion*

NOTCH inhibitors have great potential because they may target the most aggressive treatment resistant tumour cells, but more research is needed to generate biomarkers that should be used to select patients and follow treatment response. Only then, we can carefully assess the real potency of NOTCH pathway inhibitors in patients. We are not(ch) there yet.

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