NON-CONFIDENTIAL

Identification and in vivo testing of pharmacological inhibitors of essential Fra-1 target genes for breast cancer metastasis

Although recent medical and scientific advances have increased survival rates of breast cancer patients dramatically, therapeutic improvement is still invaluable for making the next step in the treatment of breast cancer and in particular breast cancer metastasis.

In order to discover novel drug targets that can improve the treatment response of currently used anti-breast cancer agents, we have previously developed a Fra-1 gene expression signature that can accurately predict breast cancer recurrence (Desmet C., Gallenne, T., *et al.*, PNAS, in press). This prognostic classifier also rendered us able to discover Fra-1 target genes that are highly correlated to poor prognosis, and thus are essential for disease progression. Our systematic investigation of these genes proved that the use and/or development of small-molecule inhibitors against the gene products might be beneficial for clinical outcome, based on the effects we observed in mouse models of breast cancer metastasis.
We have identified at least two genes whose genetic inhibition has a strong inhibitory effect on breast cancer metastasis. They have proven to be valuable leads towards the development and use of potential new anti-breast cancer compounds.

First, we discovered that the Adenosine A2b receptor (ADORA2B) is essential for the metastatic phenotype of breast cancer cells. In this ongoing project we have shown that a commercially available (currently non-cancer-related) drug against ADORA2B was able to enhance the effect of a common chemotherapeutic agent (docetaxel) to suppress breast cancer metastasis (Figure 1). More recently, we have unravelled the molecular basis underlying these observations, by demonstrating that inhibiting ADORA2B leads to a strong decrease in the formation of filopodia – cellular protrusions contributing to cancer cell migration and invasion (Figure 2) (Desmet, C., Gallenne, T., *et al.*, PNAS, in press). This understanding enables us to more specifically aim at using inhibitory drugs for this protein in a combinatorial treatment setting, targeting multiple aspects of metastasis formation, but also primary tumour growth. Experiments focusing on these particular aspects are currently ongoing.

Experimental inhibition of the other gene, which was identified with the Fra-1 classifier and is as yet unknown to have a key role in breast cancer, strongly inhibited not only metastatic spread, but also primary tumour growth of breast cancer cells *in vivo*.

Presently, there are no available inhibitors against its protein. In order to develop such a compound we have initiated a collaboration with MRC Technology UK which has a team of multi-disciplinary experts aiding us in the project. During last year's exploratory phase we have already managed to produce large quantities of recombinant protein and optimised a high-throughput assay platform that will facilitate the screening of the tens of thousands of compounds in the small molecule libraries owned by MRC-T. So far, a pilot screen has been done with ±2000 compounds and from it a few hit-compounds already were identified. Besides that,

both parties have a multitude of *in vitro* and *in vivo* assays developed and available to further validate the screens results

In March of this year, the official project launch of the collaboration will take place in London. We have already established a good relationship with MRC-T and are in fact moving ahead of schedule. To identify small-molecule inhibitors against the protein, we are about to initiate the full-scale screen and with the results thereof we will use suitable hit-compounds in our *in vitro* and *in vivo* systems to determine the potency of this protein as a breast cancer target.

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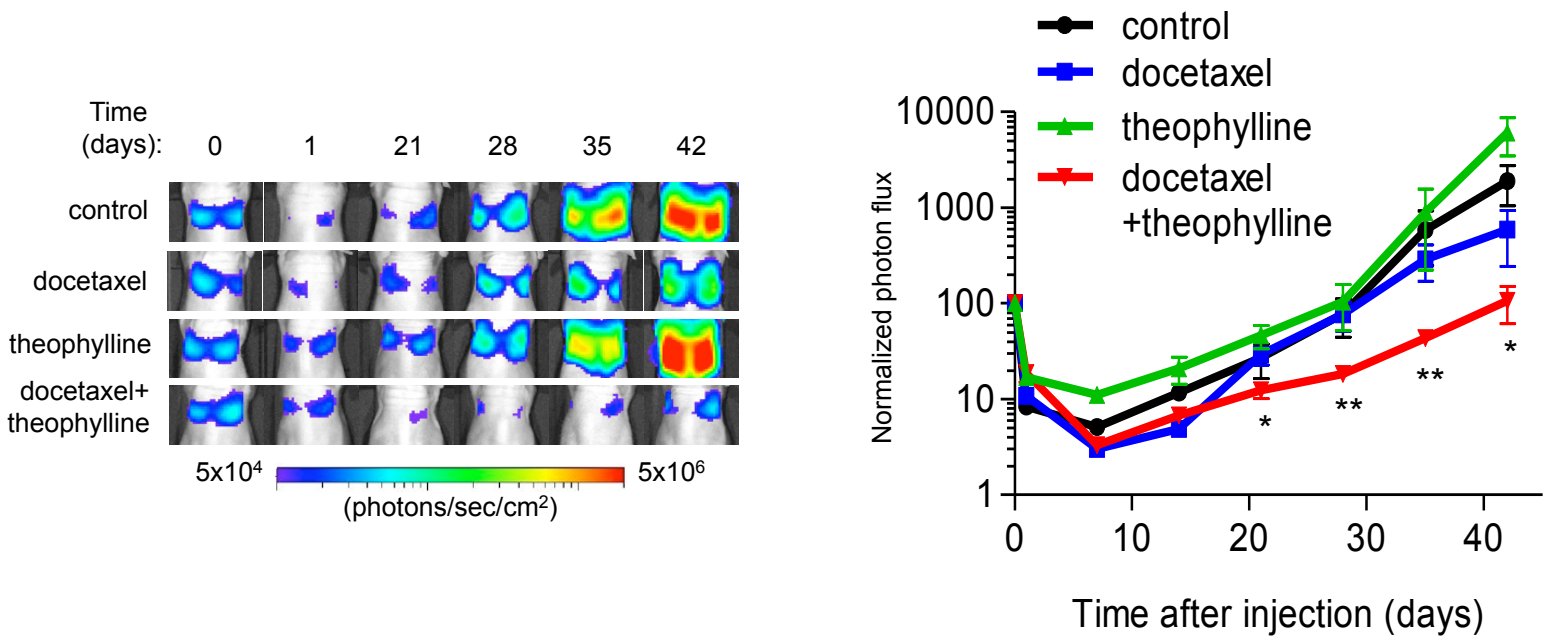

Figure 1 | The ADORA2B inhibitor theophylline can substantially enhance the effect of commonly used chemotherapeutic docetaxel in a mouse model of breast cancer metastasis.

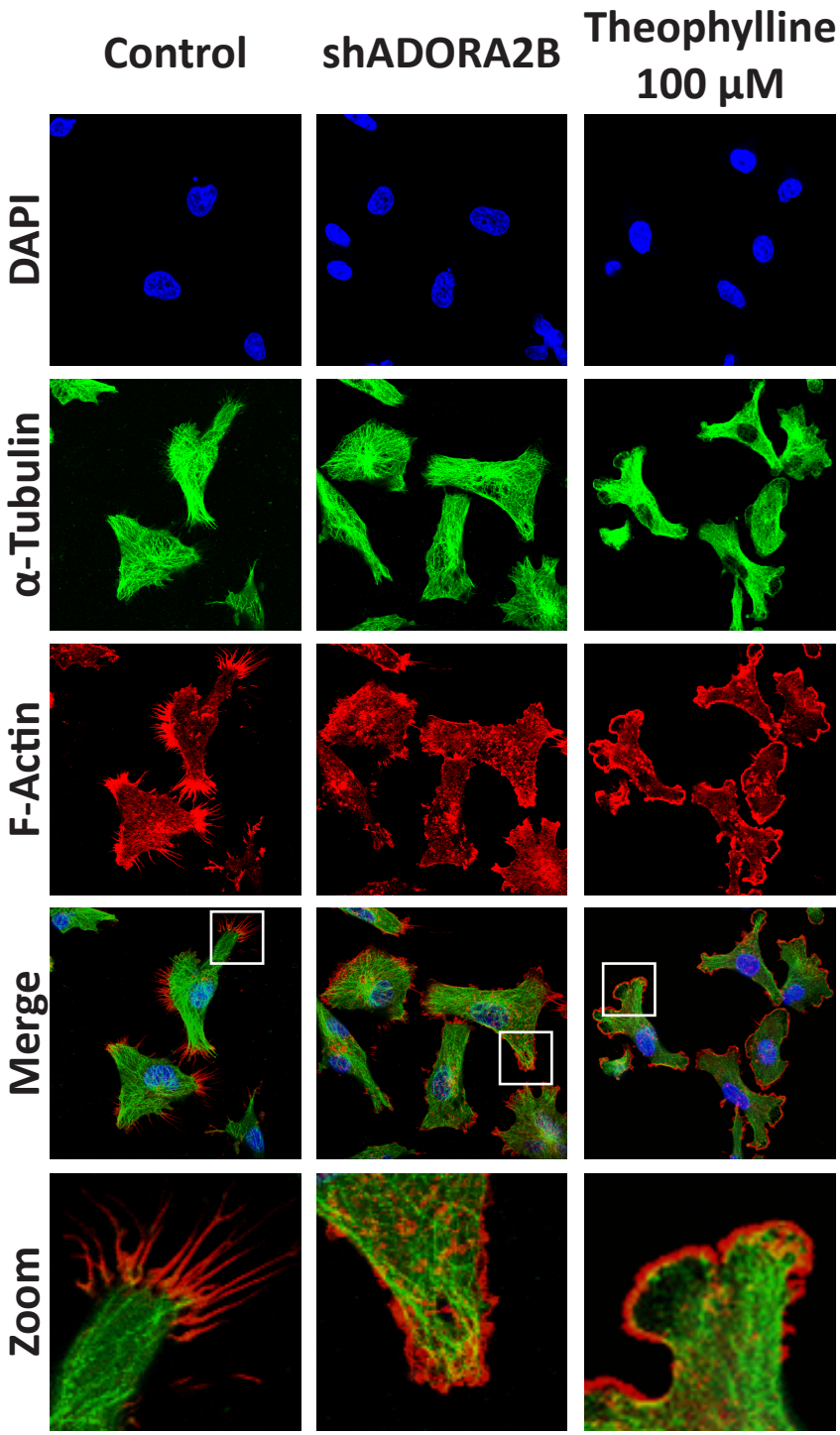

Figure 2 | Downregulation with short-hairpin RNAs and chemical inhibition of ADORA2B with theophylline both demonstrate a complete loss of filopodia formation in aggressive breast cancer cells (shown in red); these cellular protrusions involved in the cells' ability to migrate and invade are known for their roles in cancer progression.