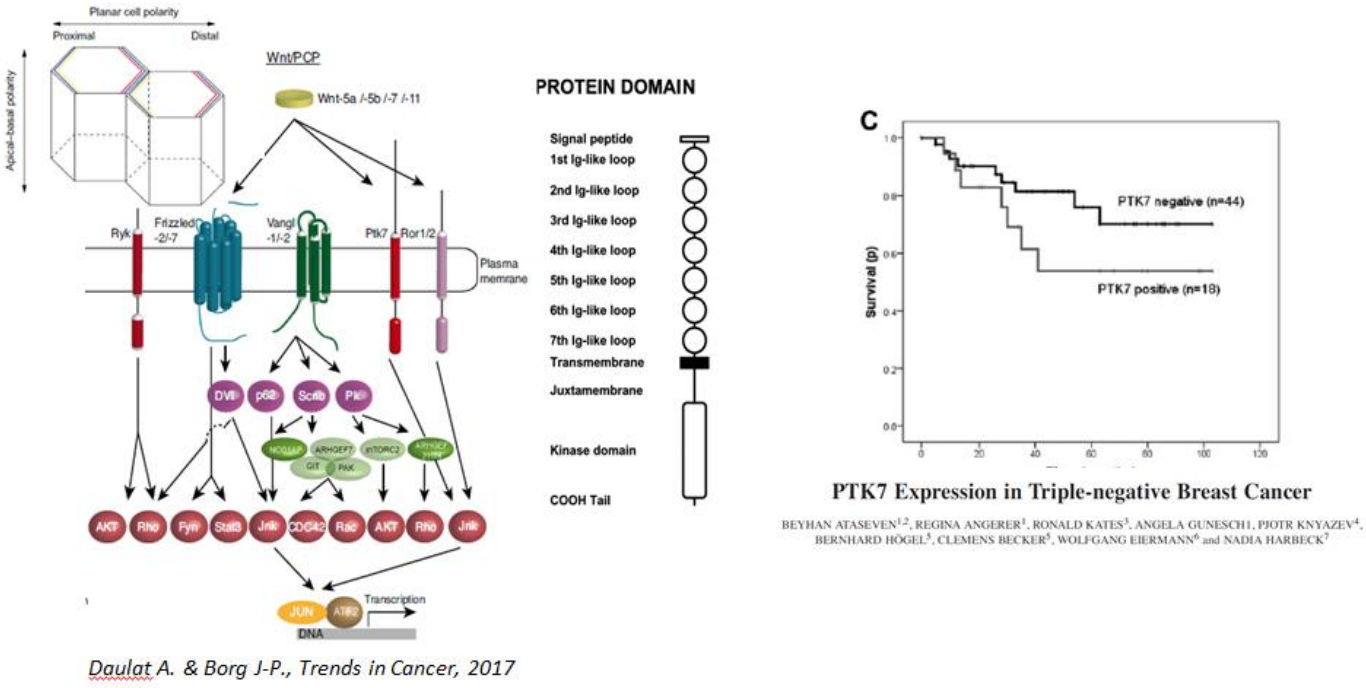
TARGETING OF THE PTK7 TYROSINE KINASE RECEPTOR IN BREAST CANCER

Authors: Laetitia Ganier (1), Stéphane Betzi (1), Carine Derviaux (1), Christophe Muller (1), Philippe Roche (1), Xavier Morelli (1), Jean-Paul Borg (1)

(1) Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm, CNRS, Institut Paoli-Calmettes, Marseille, F-13009, France, Aix-Marseille University, Marseille, France

Breast cancer is a major public health issue and impact 1 for 9 women during their life. Breast cancer is a heterogeneous disease and four main subtypes can be described associated to different clinical behaviours. Among those, basal or triple-negative breast cancer (TNBC) is a very aggressive subtype with poor prognosis outcome due to lack of targeted therapies (1).
Deregulation of cell polarity is a hallmark of cancer in solid tumors that participates to tumor development. The alteration of tissue organization and its homeostasis results in perturbation of signalling pathways. In breast cancer, cancer cells frequently reactivate developmental pathways including the Wnt/Planar cell polarity (PCP) signalling pathway which plays an important role during tumorigenesis. PTK7 is a membrane receptor belonging to the tyrosine kinase receptor superfamily and is involved in Wnt/PCP signalling regulation (2,3). However, PTK7 is described as a pseudokinase due to a lack of kinase activity. PTK7 overexpression was found associated to poor prognosis and has been also involved in resistance to chemotherapy in basal breast cancer (4) rendering PTK7 a promising new therapeutic target (Figure 1).

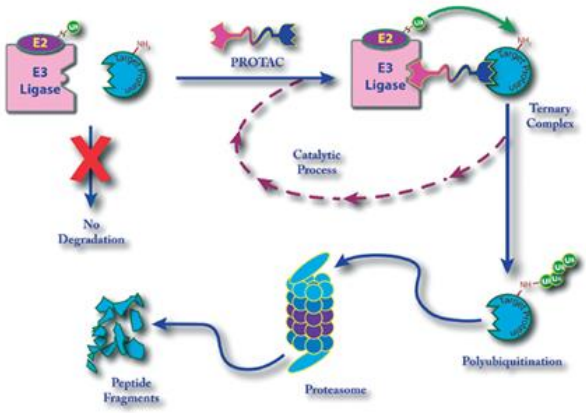


Daulat A. & Borg J-P., Trends in Cancer, 2017

Figure 1 : PTK7 - a membrane receptor involved in Wnt/Planar cell polarity signalling pathway and associated with poor prognosis in TNBC

This project aims to offer an alternative to therapeutic monoclonal antibodies against PTK7 which have already moved into clinical development (5) and is based on the discovery of chemical compounds specific to the PTK7 kinase domain. Because of the lack of enzymatic activity, we decided to eliminate PTK7 expression in cancer cells with compounds using the Proteolysis-Targeting Chimera (PROTAC) technology which induces the degradation of the target (Figure 2).

Figure 2 : Proteolysis Targeting Chimera - This strategy use small bifunctional chemical compounds that bind firstly to the targeted protein and secondly to an E3 ubiquitine ligase able to address the protein of interest for degradation by the proteasome. The first two PROTACs entered into clinical studies this year.

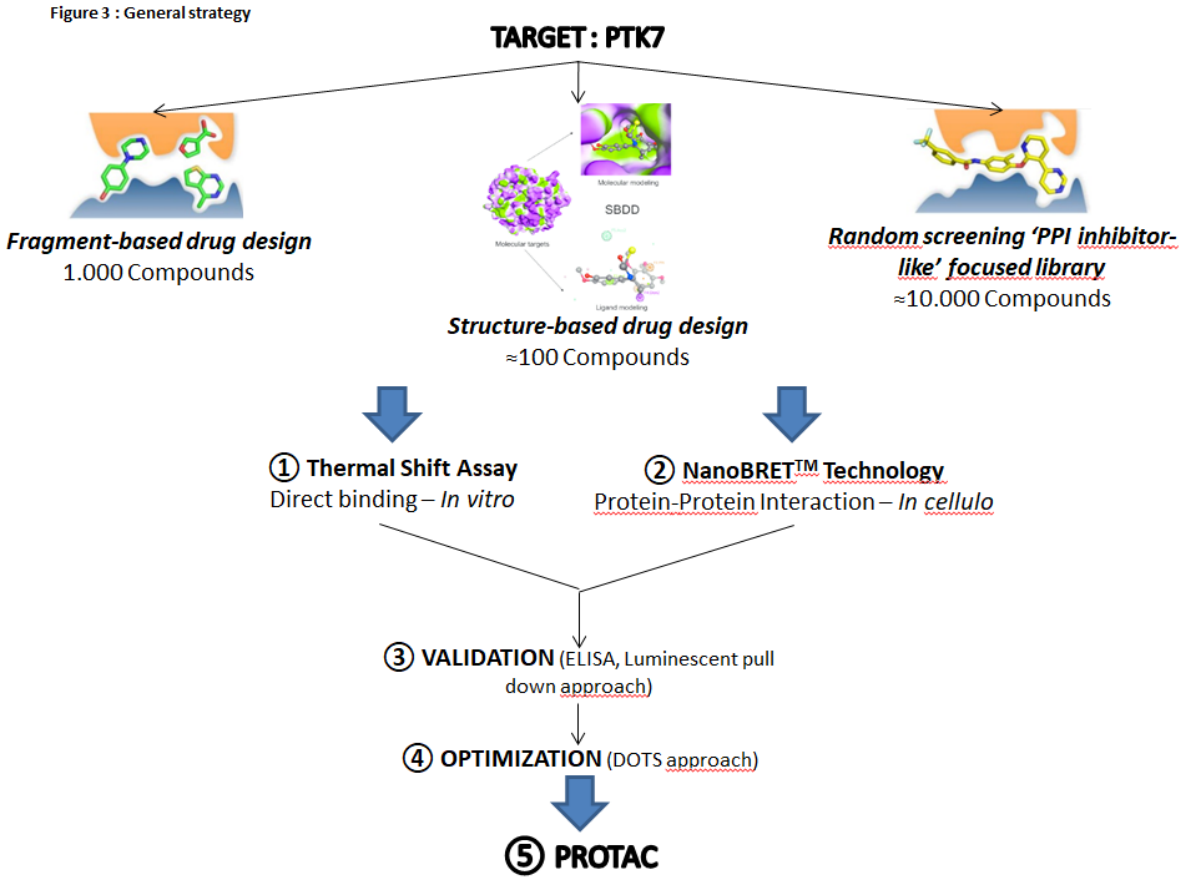


Small molecule-mediated protein knockdown as a new approach to drug discovery†

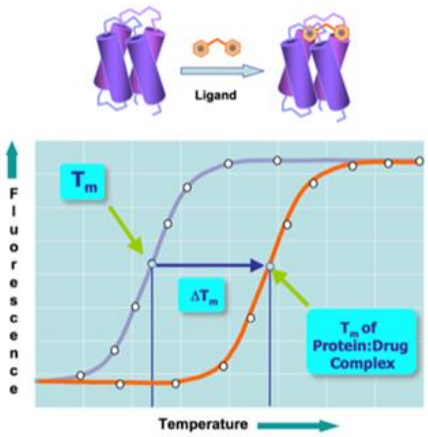
Christopher P. Tinworth,[ab] Hannah Lithgow[ab] and Ian Churcher*[a]

In order to identify chemical compounds that interact specifically with PTK7, we have developed different screening strategies. Several chemical libraries have been screened *in vitro* using Thermal Shift Assay as a first approach. In parallel, we also developed a second screening strategy *in cellulo* based on the NanoBRET™ technology for which the development of the assay was performed by Promega (Figure 3/4).

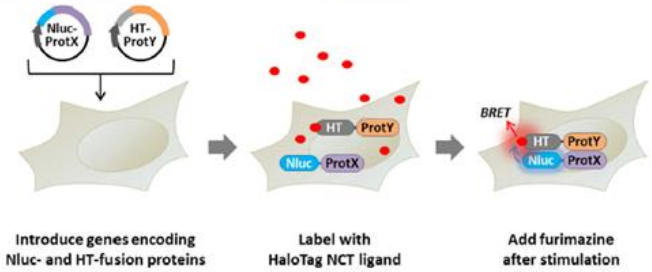
Figure 3 : General strategy



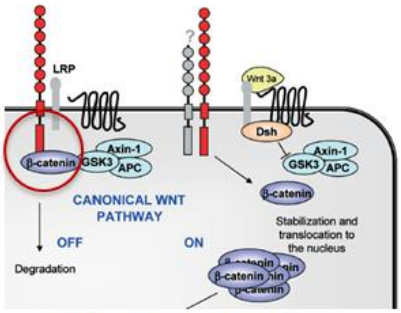
① Thermal Shift Assay
Direct binding – *In vitro*



② NanoBRET™ Technology
Protein-Protein Interaction – *In cellulo*



Machleidt et al., ACS Chem. Biol., 2015



Lhoumeau et al., J Cell Cycle, 2011

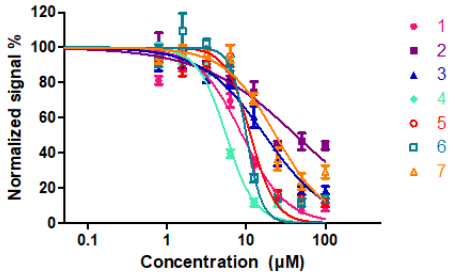
Figure 4 : Assays principle - 1/ Thermal shift assay allows quantification of the variation in thermal denaturation temperature of the targeted protein with different potential ligands *in vitro*. 2/ The NanoBRET technology allows the detection of protein interactions *in cellulo* at low levels and is applicable for drug screening. In this project we decided to develop this assay against the PTK7:βcatenin interaction

Selected 'hit' compounds will be validated using biophysical orthogonal methods (Figure 5) and optimized by medicinal chemistry. In this first objective, we will also evaluate chemical derivatives specific for E3 ubiquitin ligases. The second objective will be to further assess the efficacy of the identified compounds *in vitro* and *in vivo* using basal breast cancer cell models.

Promega

A

	1	2	3	4	5	6	7
IC50	8.911	42.31	17.17	5.566	11.36	10.04	23.46



B

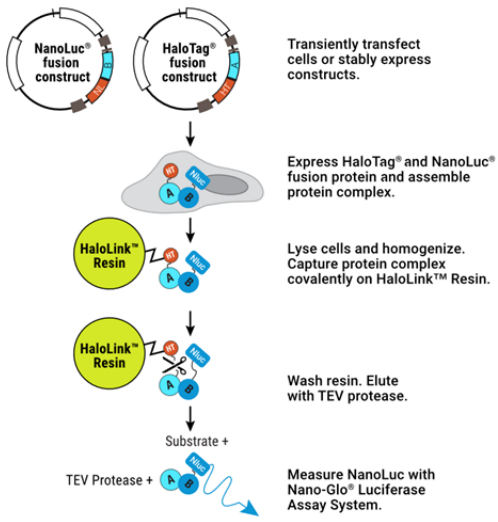


Figure 5 : A/ Selected compounds from the NanoBRET screening (over 10.000 compounds) with IC50 evaluation
B/ Selected 'hits' compounds will be validated using a luminescent pull-down approach also developed by Promega

Reference(s)

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