Dear reader,

I did not get the time to make a video. I hope the text below helps explain the poster…

Wnt-11 as a potential target for therapy in prostate cancer.

There is increasing evidence that Wnt signaling plays a role in prostate cancer, particularly in treatment-resistant and metastatic disease. Progress is being made targeting Wnt signals. However, the drugs that have been developed thus far have some potential drawbacks. Porcupine inhibitors block secretion of all nineteen Wnts, including those that may be beneficial, like Wnt-5a, which has been found to promote tumour cell dormancy in bone. Targeting Wnt receptors also impacts signals from multiple Wnt ligands because of receptor redundancy. Moving closer to the nucleus, there are drugs that block beta-catenin interaction with Tcf/LEF family transcription factors. These can block tumour growth but may not affect so-called non-canonical Wnt signals, which do not act through beta-catenin, at least not directly. As Wnts are short-range secreted proteins, they can be readily targeted using antibodies. One possible solution, therefore, is to generate antibodies that block each Wnt family member and use them in combination, tailored to the patient Wnt expression profile of the tumour, which is likely to change over time.

We have focused on Wnt-11, a non-canonical Wnt. The **1st panel** of the poster is a combination of published data that I hope makes the case for Wnt-11 as a target for therapy in prostate cancer. In prostate cancer patients Wnt-11 is elevated in around 25% of primary tumours and particularly high in metastases (not shown). There is also an association between high WNT11 gene expression and biochemical relapse and disease-free survival.

WNT11 levels are increased by many signals, but the effect of androgens is particularly interesting. In LNCaP cells, WNT11 expression is repressed by androgens, whereas in VCaP cells, which express ERG, androgens further increase the already high basal levels of Wnt-11. Intrigued by this differential regulation, we looked at Wnt-11 and ERG in adjacent sections of patient tumours. Representative images are shown in the **2nd panel**. We found no correlation, suggesting Wnt-11 is regulated by other or additional factors. These may include hypoxia, which induces Wnt-11 in some other cancer cell types. There is however a strong correlation between ERG and the Wnt-11 receptor FZD8, suggesting paracrine Wnt-11 signals from ERG-negative cells might further activate Wnt signals in ERG-positive cells.

To target Wnt-11, we developed monoclonal antibodies to a series of Wnt-11-derived peptides. The strategy and key results are shown in the **3rd panel**. Two antibodies, E8 and F10, were selected because they recognised Wnt-11-expressing cells by immunocytochemistry. Both turned out to recognise the same peptide but have slightly different properties. They do not blot well, but they are able to bind to Wnt-11 associated with the cell surface. The blot on the left is of extracts from cells FACS sorted using the E8 antibody and probed with a commercial Wnt-11 antibody.

E8 and F10 do not significantly affect prostate cancer proliferation in 2D cultures, but do inhibit growth of 3D spheroids. With respect to Wnt signalling, the antibodies reduce phosphorylation of Dvl-2, as shown on the top right of this panel.

Consistent with our gene silencing studies, the antibodies inhibit prostate cancer migration and invasion. Inhibition of invasion is similar to that observed for the porcupine inhibitor C59.

We have also observed that WNT11 is induced in PC3M cells using the hypoxia mimetic DMOG, as shown in the western blot on the bottom right. DMOG was found to increase PC3M migration and E8 inhibits the effect of DMOG.

The **4th panel** summarises our results so far on the *in vivo* effects of the antibodies. We observed a significant reduction in PC3 cell tumour size in chick CAM assays using the E8 antibody. We also outsourced some orthotopic xenograft assays with PC3M cells. The timing of antibody treatment in that study was not ideal but we nevertheless observed a trend for a reduction in metastasis using the E8 antibody.

The **5th panel** summarises some results where we deleted the antibody epitope in Wnt-11. The mutated protein, called AE, for antibody epitope mutant, is expressed on the cell surface to a similar extent as wild-type Wnt-11. However, the AE mutant is unable to promote PC3 cell migration. In addition, Wnt-11 and FZD8 additively activate the noncanonical Wnt ATF2-luciferase reporter but the AE mutant does not. Also, while Wnt-11 inhibits Wnt-3a activation of a beta-catenin/Tcf gene reporter, the AE mutant does not. These results suggest to us that the antibody epitope plays a role in Wnt-11 interactions with its receptors, something we are currently exploring.

While Wnt-11 is in our opinion an excellent therapeutic target in a subset of prostate cancer patients, other Wnt proteins may also play a role. We have therefore explored the possibility that the same region might also be important for activity in another Wnt by mutating Wnt-3a. Wild-type and mutant Wnt-3a were expressed at similar levels on the surface of transfected cells. However, in contrast to wild-type Wnt-3a, mutant Wnt-3a shows very low activity in gene reporter assays. We are planning to develop antibodies targeting this same region in multiple Wnt proteins, focusing on those Wnt that are upregulated in prostate cancer.