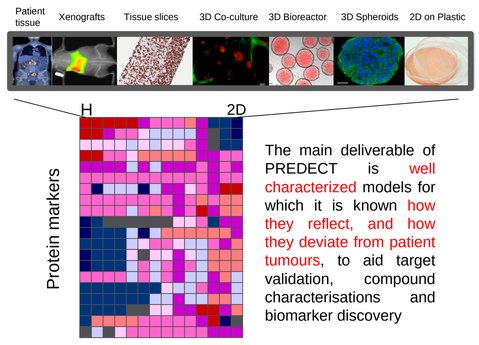
Generation of 3D models that more faithfully represent prostate cancer tumour complexity

Suzana Vidic[1,2], Malin Åkerfelt[3], Ronald de Hoogt[2], Ingrid Verberne[2], Sigrun Erkens[1], Yolanda Chong[2], Michael Barbier[2], Varda Rotter[4], Ralph Graeser[2], Matthias Nees[3], Wytske van Weerden[1]

[1] Erasmus MC, Rotterdam, The Netherlands; [2] Janssen Pharmaceutical Companies of Johnson and Johnson, Beerse, Belgium; [3] VTT Technical Research Centre of Finland, Turku Finland; [4]Weizmann Institute, Rehovot, Israel

BACKGROUND AND AIM

- 96% of oncology drugs fail clinical trials, a majority due to lack of efficacy.
- Only 1/3 of the cost of a drug is spent in pre-clinical research
- PREDECT aims to develop and characterise *in vitro* models that more closely reflect patient tumour biology.
- Platforms developed include (humanised) xenografts, GEMMs, tissue slices, 3D and 2D cell culture models.
- Complex 3D cultures incorporate more components (extracellular matrix, stromal cells) of a native tumour environment than classical 2D cultures.
- Within WP2 (prostate) of PREDECT, we develop 3D cell culture models growing PC (LNCaP, PC346c, VCaP) and stromal cells (WPMY-1, CAF) in extra cellular matrices (BME, collagen I, and a mix thereof)
- The cultures are monitored while growing as well as fixed and paraffin-embedded.
- IHC of FFPE cultures allows for cross-comparison with other PREDECT platforms



TMA and Imaging

CONCLUSIONS

Our 3D culture growth analysis revealed differential roles of stromal cells and extracellular matrix on tumour cell growth behaviour:

- WPMY stimulated LNCaP growth and vice versa in BME, but not collagen-I.
- CAFs did not affect LNCaP growth, but a co-stimulatory effect was observed in collagen-I with PC346C.
- Tumour – stroma cell ratios and density were important for the observed enhanced growth

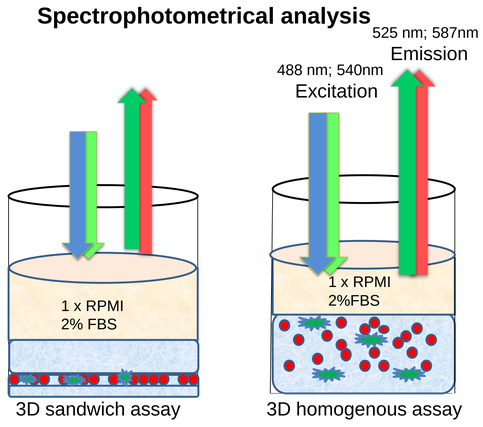
Confocal imaging showed inter- and intra heterogeneity of 3D co-cultures:

- Not all spheroids, and not all cells within a given spheroid expressed AR
- Ki67 staining indicated high proliferation rates at the periphery of the spheroids
- CAFs shape and behaviour was affected by the matrices

Molecular analyses (u-array, cytokines) are planned, and the comparison of the FFPE 3D culture to the other PREDECT platforms as well as patient samples are underway.

MATERIALS AND METHODS

3D Cell Culture Growth Assays

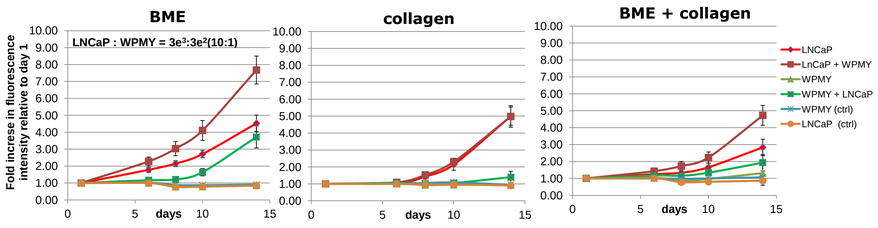


+	reproducible and screenable assay	+	reproducible and screenable assay
+	close cell to cell contacts result in intensive cell crosstalk	-	larger distances between the cells may result in weaker cell crosstalk
+	all cells in one plane allows for efficient real-time confocal imaging	+	'real' 3D growth in all dimensions
-	but limits the preparation of more histological sections needed for TMA	+	thickness of matrix allows preparation of many histological sections used for TMA

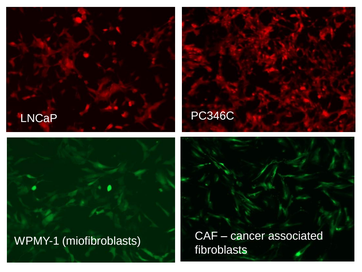
- Tumour cells : **LNCaPs or PC346C**
- Stromal cells: **WPMY-1 or CAFs**

LNCaP and PC346C PC cells were embedded, with or without WPMY-1 or CAF stromal cells, in BME, Collagen I, or a 1:1 BME-Collagen mix, and the growth of red and green fluorescent cells was monitored by spectrofluorometer.

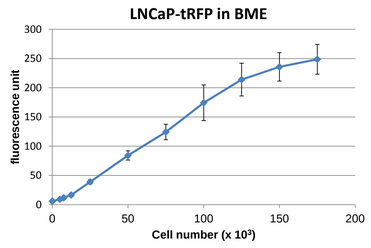
Matrices affect the growth of tumour-stroma cells in 3D co-cultures



3D sandwich assay - growth of LNCaP-tRFP and WPMY1-eGFP in BME, collagen I, 1:1 mixture of both, measured using corresponding filter sets. LNCaP cells grew as a 3D monoculture in all three matrices tested. In monoculture, not much growth of WPMY-1 was observed. In co-culture and in presence of BME, there was a growth stimulatory effect of WPMY on LNCaP, and vice versa. No bleed through was detected when WPMY1 were measured in red channel (WPMY ctrl), and vice versa (LNCaP ctrl).

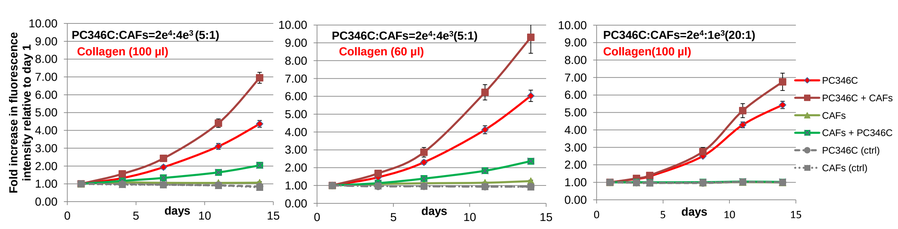


Prostate cancer cells and fibroblasts were transduced with tRFP and eGFP lentiviral constructs, respectively. Stably transduced cells were FACS sorted for uniform fluorescence.



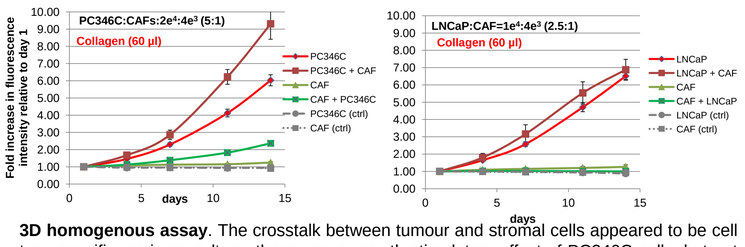
Calibration curve showing the correlation between red fluorescence and cell number.

Ratio and distance between tumour and stromal cells affect the cell growth of 3D co-cultures vs. 3D monocultures



3D homogenous assay – growth of PC346C-tRFP and CAF-eGFP in collagen. Green and red fluorescence were monitored in 3D monoculture and 3D co-culture. Reduction of matrix volume or the ratio between tumour and stromal cells resulted in increased growth rate of 3D co-cultures, suggesting a critical crosstalk between tumour and stromal cells.

Crosstalk between tumour and stromal cells is cell type specific



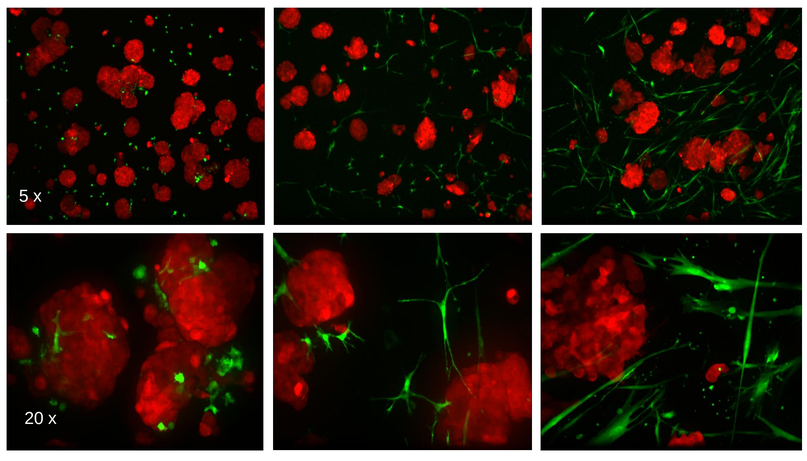
3D homogenous assay. The crosstalk between tumour and stromal cells appeared to be cell type specific, as in co-culture, there was a growth stimulatory effect of PC346C cells, but not LNCaP cells, on CAFs and vice versa.

Live and fixed-cell confocal imaging

Spinning disc confocal
•Zeiss Axiovert-200 M microscope with Yokogawa CSU22 spinning disc confocal unit

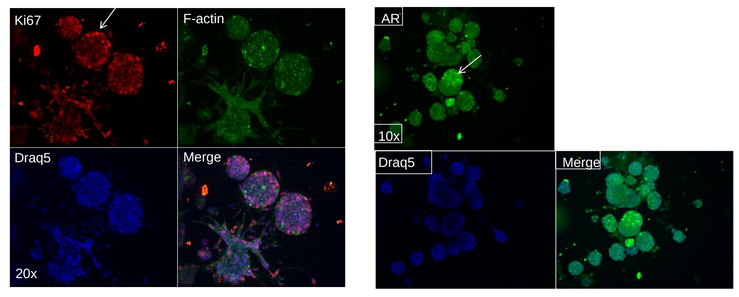
3D imaging (live or fixed)

Matrices affect the spreading of fibroblasts



Live-cell imaging of 3D co-cultures **LNCaP-dsRed and CAF-GFP (2:3**) growing in matrigel or collagen or matrigel-collagen mixture (1:1). CAFs appeared to differentiate only in presence of collagen.

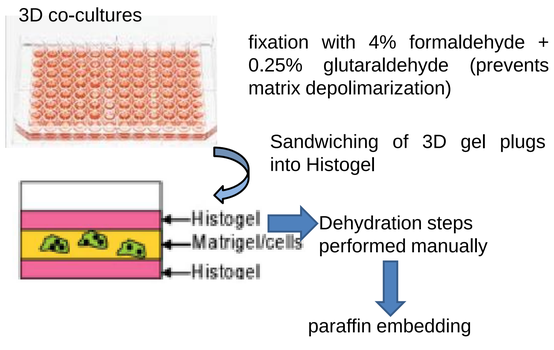
Inter- and intra-spheroidal heterogeneity



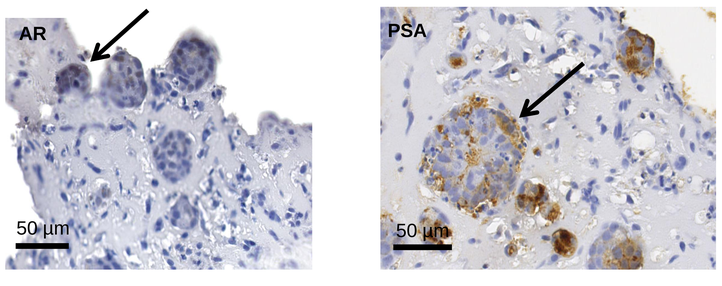
Immunofluorescent stainings and imaging of 3D co-culture LNCaP-dsRed and WPMY-GFP (10:1): Ki-67: (cell proliferation), F-actin (cytoskeleton), Draq5 (living cells), AR (androgen receptor).

PREPARATION OF 3D CULTURES FOR TMA

Paraffin embedding and immunostaining of 3D co-cultures



Expression of prostate specific molecules in 3D co-cultures of prostate cancer



Immunostaining of paraffin embedded samples LNCaP-tRFP: WPMY-eGFP (10:1) in BME matrix

ONGOING AND FUTURE ACTIVITIES

- Perform similar 3D-culture assays under hypoxic conditions (1- 3% O_2)
- Use of standardized 3D co-culture assay conditions to include castration resistant prostate cancer (CRPC) clones (+/- WPMYs, CAFs).
- Perform 3D culture assays with matrix-embedding of pre-formed 3D aggregates for cell lines that do not grow as spheroids, e.g. VCaP.
- Confocal imaging and immunohistochemical analysis of 3D cultures, and comparison with primary tumour samples in TMAs will reveal which model(s) and conditions most closely resemble in vivo prostate cancer tissue.