Patient-Derived Xenograft Screening in a Three-Dimensional Tumor Growth Assay incorporating Stromal Elements to Recapitulate the Human Tumor Microenvironment

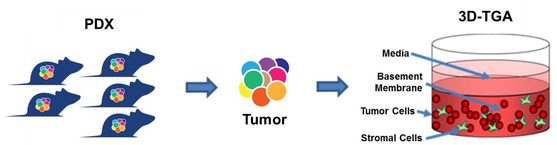
Abstract No.5033

Diandong (Simon) Jiang, Jane Wrigley, Sumanjeet Malhi, Jamie Wood, Stephanie Morton, Louise Wainwright, Lucy Harris, Jason King, Yinfei Yin, and Rajendra Kumari
Crown Bioscience UK, Ltd, Loughborough, UK

Visit us at Booth #3012

INTRODUCTION

Preclinical *in vitro* and *in vivo* tumor models lack human stromal cells and are inadequate for the assessment of novel tumor microenvironment targeted therapies. Here we report on a 3D-tumor growth assay (3D-TGA) in which patient-derived tumor cells are admixed with a basement membrane extract and human bone marrow-derived mesenchymal stem cells (bmMSCs) in a 96-well format for screening. Response to standard of care (SoC) agents such as paclitaxel, and targeted agents such as erlotinib, were compared to subcutaneous xenograft responses *in vivo*.



METHODS

3D-TGA: Tumor cells or xenograft material is disaggregated to yield a pure epithelial population, then admixed and suspended in basement membrane extract (Cultrex®, Trevigen) with and without bmMSCs. Cells were maintained for 3 days before they were treated with a panel of test agents, such as paclitaxel, AZD2014, erlotinib, or carboplatin, and final cell viability was determined on Day 7 by alamarBlue® assay. IC_{50} curves were determined using GraphPad Prism 6.0.

In vivo: Subcutaneous tumor growth in nude mice was evaluated 3 times a week by measuring the tumor in two dimensions using electronic calipers for the duration of the study, and tumor volumes were estimated using the formula 0.5 (LxW^2). PDX were co-implanted with human MSCs and treatment was initiated when tumor growth was established (approx. 150-200mm^3).

Table 1. Summary of NSCLC PDX models

Model	Cancer Type	Subtype	EGFR	FGFR (CN)	TP53	LKB1
LU6402	NSCLC	SCC	WT	9	WT	WT
LU6404	NSCLC	SCC	WT	6	NO	WT
LU6411	NSCLC	SCC	WT	3	NO	F354L
LU6422	NSCLC	ADC	L858R	NO	WT	NO

RESULTS

The models in Table 1 were optimized for the 3D-TGA assay. Xenograft tissue was used for either 3D-TGA or *in vivo* study. The response to SoC and targeted agents is shown in Figure 1 and 2 for both 3D-TGA and *in vivo* study, with IC_{50} values summarized in Table 2.

Table 2. Summary IC_{50} values for NSCLC PDX models

Model	IC_{50} (μM)				
	Paclitaxel	AZD2014	Erlotinib	Pemetrexed	Carboplatin
LU6402	3.1	1.4	~25	N/A	127.8
LU6404	0.6	1.3	>10	N/A	76.8
LU6411	N/A	N/A	3.5	1.6	72.5
LU6422	N/A	N/A	4.3	>10	23.5

Fig 1. Efficacy of paclitaxel in combination with AZD2014 in the LU6402 PDX model. (A) *In vivo* results; **(B)** *ex-vivo* 3D-TGA with/without bmMSCs**.** 3D-TGA with bmMSCs shows corresponding response of the combination response when compared to *in vivo*

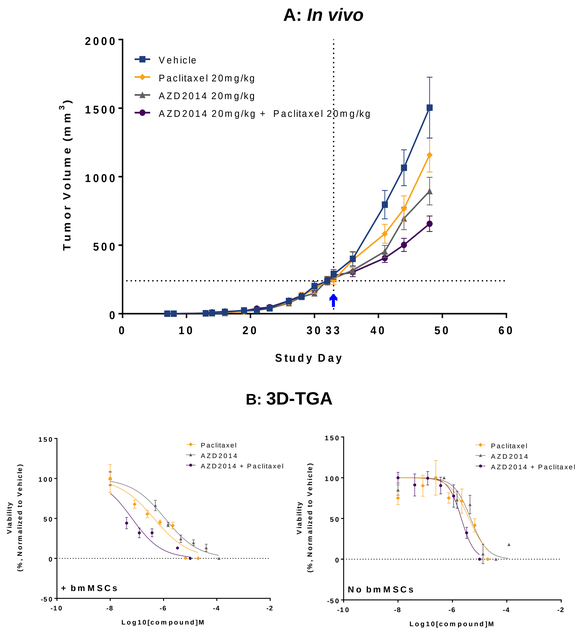
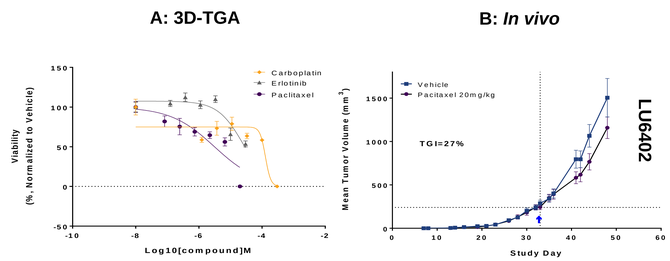
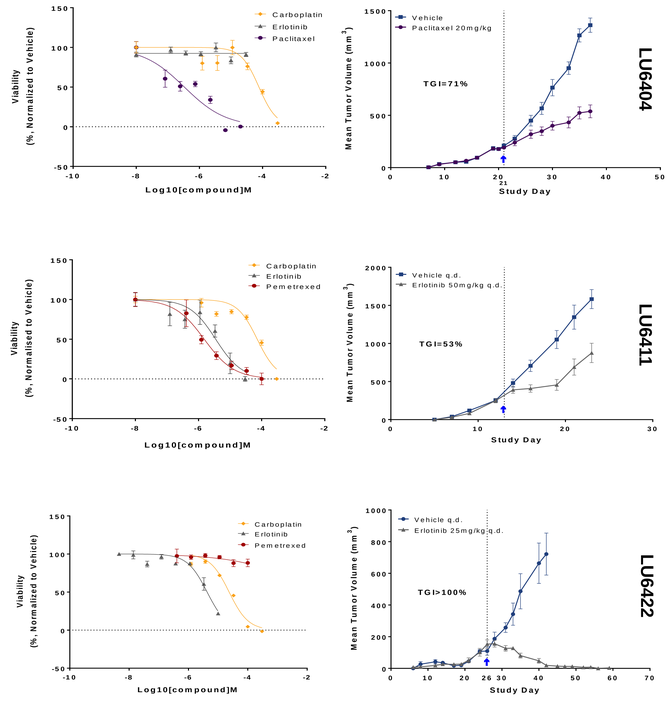


Fig 2. Efficacy of a panel of SoC or targeted agents (e.g. erlotinib) in (A) 3D-TGA and (B) their corresponding efficacy *in vivo* xenograft data in the presence of bmMSCs





SUMMARY

- The squamous NSCLC model LU6402 was assessed with AZD2014 (an mTOR inhibitor) and paclitaxel in the 3D-TGA in the presence of bmMSCs, which showed an additive effect of AZD2014 in combination with paclitaxel on tumor cell growth
- A similar efficacy was observed in a subcutaneous *in vivo* xenograft; however, a combination finding was not evident when the stromal cells bmMSCs were absent
- The data illustrates that comparable sensitivities to various test agents between 3D-TGA and *in vivo* xenograft studies was observed
- The ADC NSCLC model LU6422, which harbors a heterozygous mutation in the EGFR intracellular kinase domain (c.2573T>G (L858R)) showed exquisite sensitivity to erlotinib in both 3D-TGA and xenograft studies
- The potency of SoC agents in the 3D-TGA is correlated to *in vivo* efficacy data
- For example, paclitaxel showed a higher potency in the 3D-TGA with LU6404 than LU6402, and a greater tumor inhibitory effect of paclitaxel was also observed in LU6404 when compared with that in LU6402

REFERENCES

Onion *et al*. Three-Dimensional, Humanised Tumour Growth Assays with Low Passage Tumours Provide a Patient- Relevant Screen for Novel Chemotherapeutics. *Mol Cancer Ther* 2016;15(4): 753-756.