

Introduction

Evidence is provided that resistances to targeted therapy drugs in non-small cell lung cancer (NSCLC) are based on substitutions for inhibited pathways and on crosstalk of generic intracellularly connected downstream pathways. Therefore, combined targeted therapies against selected pathways of this Cellular Tumorigenic Network may overcome drug resistances (Fig. 1).¹⁻³

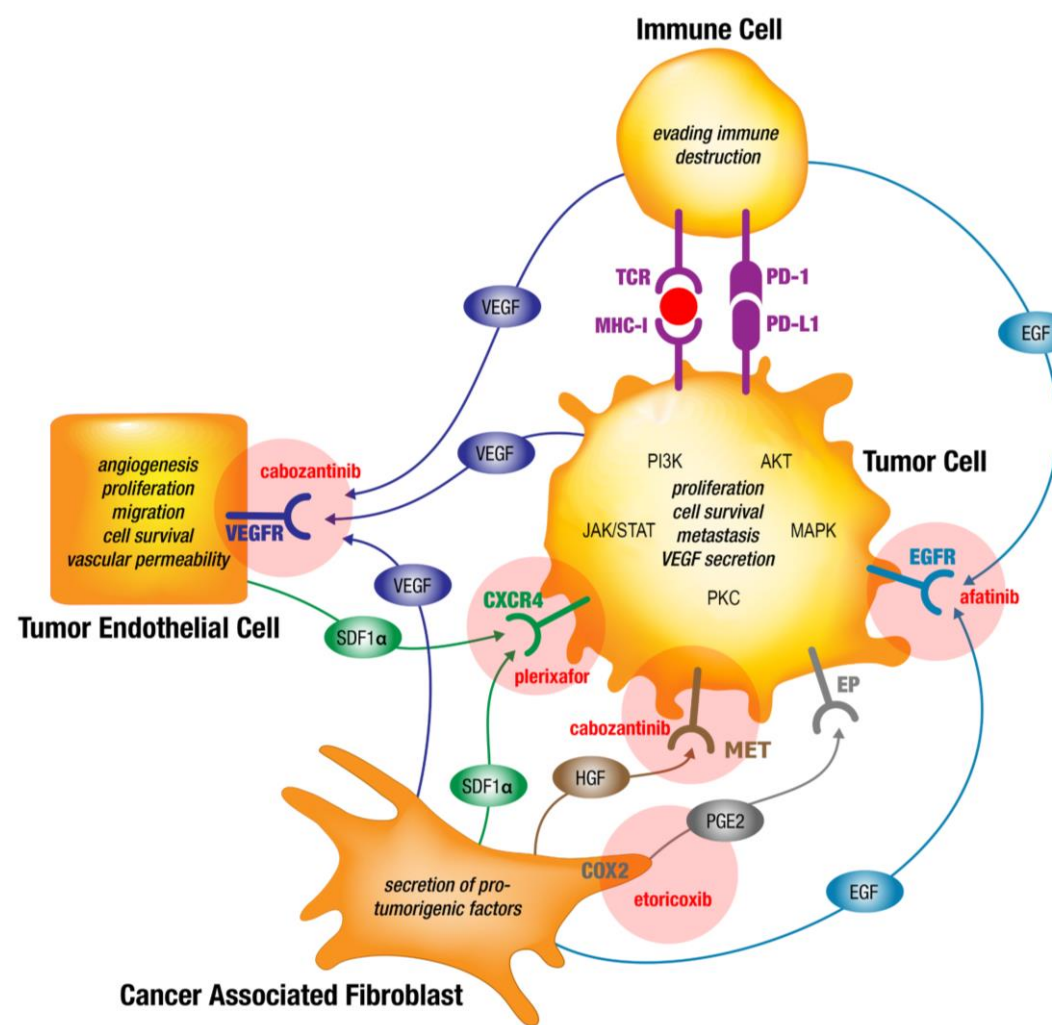


Fig. 1 Model of the chosen combination therapy regimen targeting the Cellular Tumorigenic Network

Methods and Materials

From an established PDX panel, 38 clinically well characterized patient-derived, human NSCLC tumor xenograft models (PDX) were analyzed for mRNA expression of (i) paracrine signaling pathways mediating intercellular interdependency within the Cellular Tumorigenic Network that were (ii) non-overlapping and have been described (iii) as relevant for tumor proliferation previously and druggable by approved inhibitors. Specifically, relative mRNA expression levels of **EGFR**, **EGF**, **VEGFR2 (KDR)**, **VEGFA**, **CXCR4**, **SDF-1α (CXCL12)**, **MET**, **HGF**, and **COX2** were analyzed. **TP63** was analyzed as control of the biological validity of this data set.

For experimental validation of the hypothesis, five highly resistant patient derived NSCLC PDX models were selected from this panel and subjected to treatment with a low-dose drug combination regimen of cabozantinib (15mg/kg), afatinib (15mg/kg), etoricoxib (10mg/kg), and plerixafor (5mg/kg) with 5 days on and 2 days off treatment over 4 cycles.

Results

Gene expression analysis indicates a relationship between specific genes and treatment resistances towards standard anti-tumor therapies. All nine selected genes of the Cellular Tumorigenic Network were significantly expressed and the seven highly resistant PDX models with progressive disease (PD) identified in Cluster 1, exhibiting the highest transcript expression for **VEGFA**, **MET**, **EGFR**, **KDR**, and **PTGS2** (Fig. 2).

Fig. 2 Characterization of the NSCLC PDX panel for target gene expression, single-sample GSEA and selected PDX models for targeting the Cellular Tumorigenic Network with the combination therapy regimen.

a TP63 expression; **b** Single-sample gene set variation analysis (ssGSEA) combined with a permutation-based test; **c** Relationship between gene expression and therapy resistances in NSCLC tumors; **d** Characterization of selected NSCLC tumors

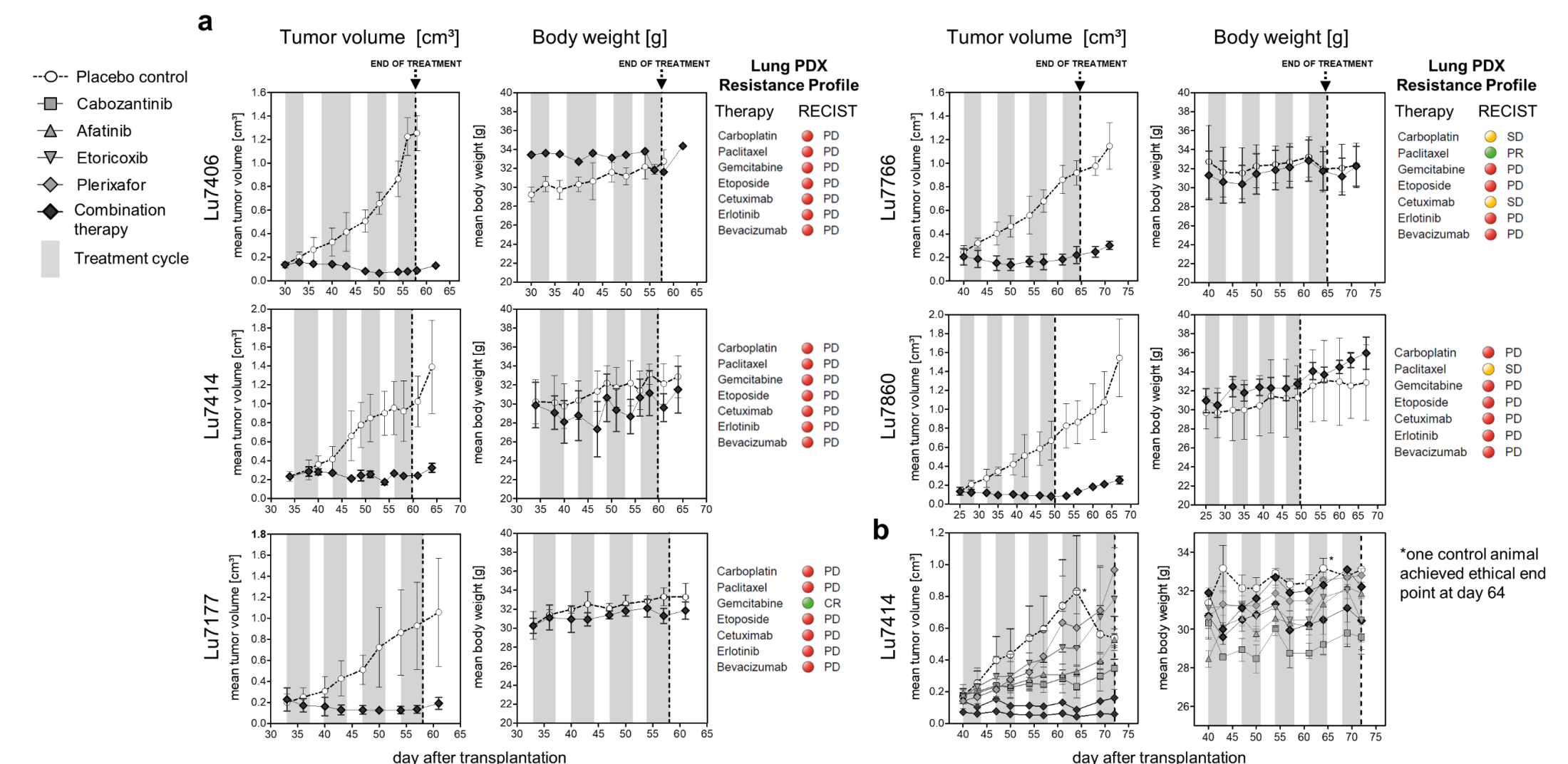
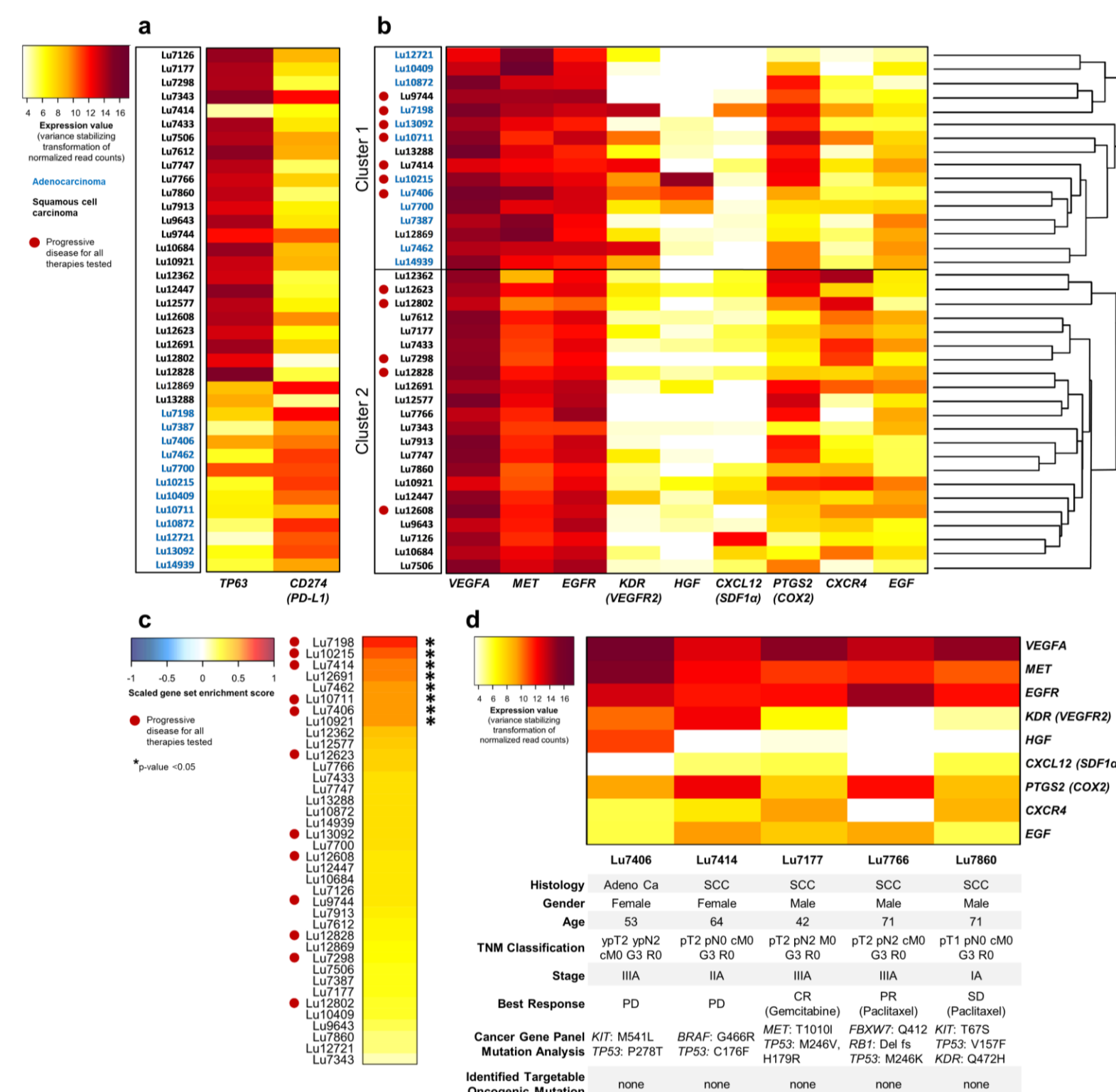
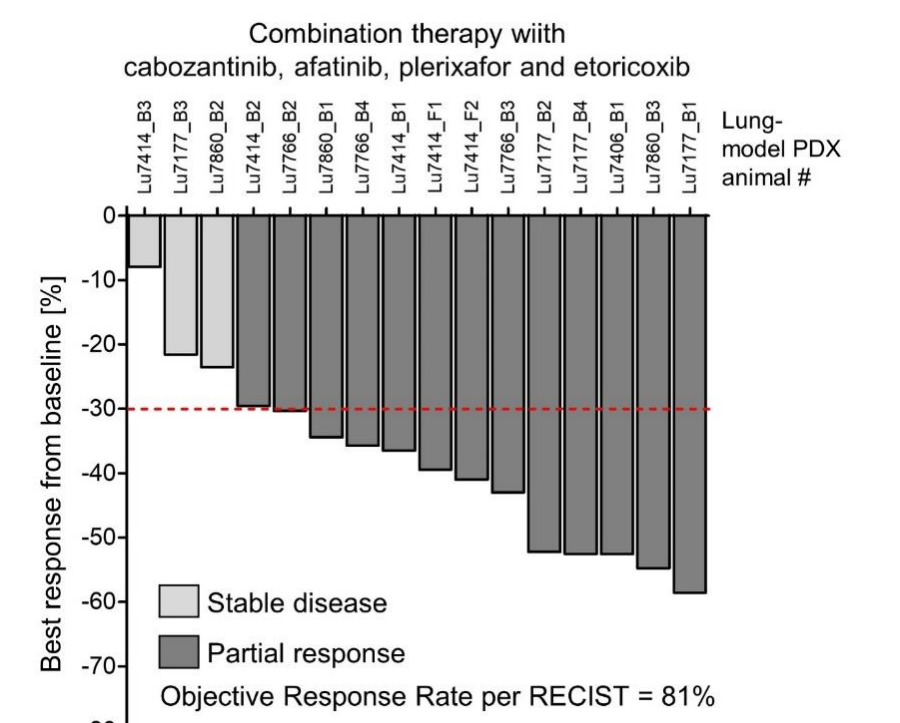


Fig. 3 Efficacy of the combination therapy regimen. **a** Treatment with the combination therapy regimen versus placebo. **b** Single compounds alone versus the combination therapy regimen. Therapy resistance per RECIST are indicated by the red circle for progressive disease (PD), yellow circle for stable disease (SD), and green circle for partial and complete response (PR, CR).

Fig. 4 Best response per RECIST analysis in all NSCLC patient-derived xenograft tumors treated with the combination therapy regimen. Depiction of all 16 NSCLC PDX tumors treated with the combination therapy as best response measured from baseline tumor size in percent. Response definition was calculated per RECIST criteria with partial response defined as 30% reduction and stable disease defined as neither response or progression in the sum of the longest tumor diameter.



Discussion and Conclusion

Analysis of gene expression profiles from the EPO NSCLC PDX panel provides evidence of a relationship between the expression of genes maintaining the Cellular Tumorigenic Network and common drug resistances which could be overcome by the drug combination regimen described here. These results strongly encourage the further validation of this combination therapy in a clinical study for advanced NSCLC patients without current therapeutic options.⁴

*Contact

Dr. Jens Hoffmann
EPO Experimental Pharmacology & Oncology, Berlin, Germany
Robert-Rssle-Str. 10, 13125 Berlin-Buch, Germany
Jens.Hoffmann@epo-berlin.com
+49 30 9489 4444

References

- Egeblad M, Nakasone ES, Werb Z. Tumors as organs: complex tissues that interface with the entire organism. *Dev Cell* **18**, 884-901 (2010).
- Hanahan D. Hallmarks of Cancer: New Dimensions. *Cancer Discov* **12**, 31-46 (2022).
- Langhammer S, Scheerer J. Breaking the crosstalk of the cellular tumorigenic network: Hypothesis for addressing resistances to targeted therapies in advanced NSCLC. *Oncotarget* **8**, 43555-43570 (2017).
- Grgen D, Conrad T, Becker M, et al. Breaking the crosstalk of the Cellular Tumorigenic Network by low-dose combination therapy in lung cancer patient-derived xenografts. *Commun Biol*. **5**, 59. (2022)