Establishment and characterization of allografts derived from a genetically engineered mouse model of non-small cell lung cancer (NSCLC)


1OncoTest GmbH, Am Flughafen 12-14, 79108 Freiburg, Germany; 2University Hospital Basel, Hebelstrasse 20, CH-4031 Basel, Switzerland; 3Institute of Pathology, Liebermeisterstrasse 8, 72076 Tübingen, Germany

Introduction
Genetically engineered mouse models (GEMM) like the KP model (DuPage et al., 2009) reflect the tumorigenesis of NSCLC in humans. KP mice carry KRAS and TP53 mutations, comparable to human tumors. Tumors arise in situ allowing immune functions, angiogenesis and inflammatory processes to interact normally with the evolving cancer cells. Typical limitations of GEMM tumors include differences in their genetic make-up to human tumors and the slow tumor development. The latter can be circumvented by grafting of primary tumors from GEMMs onto a genetically dissimilar member of the same species, a process called allografting.

Materials and Methods
KP tumors were excised from the lung of a conditionally induced genetically engineered lung cancer mouse model KP and implanted subcutaneously. When tumor growth was detected passages from animal-to-animal were performed. C57BL/6N female mice, were used to receive subcutaneous implants of the lung tumors. Furthermore, two cell lines derived from KP tumors, KP1 and KP4 (provided by Müller et al.) were injected subcutaneously and the developing tumors excised and directly re-implanted as described above. The passages were histologically, immunohistochemically (Ki67, CD31, GLUT1) and molecularly analyzed, and compared to the original in-situ tumors. Treatment experiments with Erlotinib (60mg/kg/day), BEZ235 (45mg/kg/day) and Afatinib (20mg/kg/day) in mono- and combined therapy were performed on KP1 allograft bearing mice.

Results
• Generation of subcutaneous allografts was conducted over 8 passages with stable growth of the tumors as documented by tumor-growth-kinetics and H&E staining.
• The patho-histological diagnosis for all 3 models is adenocarcinoma with papillary growth pattern and glandular distribution.
• Over the consecutive passages tumor cells seem to have acquired more pleomorphism and the number of mitoses and fibrosis increased.
• Pathohistological examination revealed no immunological reaction towards the implanted tumors.
• IHC showed different Ki-67 and CD31 expression among the 3 different models.
• The mRNA qPCR analysis demonstrated a low PIK3CA and mHER1 (EGFR) expression in KP1.
• EPCAM is highly overexpressed in the KP and KP1 and low in KP4 (qPCR analysis of cDNA data).
• BEZ235, a potent dual inhibitor of PIK3 and mTOR, revealed significant anti-tumor activity in KP1 allografts both in mono- as well as combination therapy with Afatinib.
• Erlotinib showed no significant effect in KP1 allografts, which is in line with the low mHER1 expression.

Conclusion
The generation of subcutaneous transplantable allografts from the KP GEMM was successfully conducted. The molecular analysis confirmed the similarity to human NSCLC and the original GEMM model. Allografting tumors of GEMM models seem to be an appropriate way to overcome some of the limitations of the otherwise promising GEMM model-system for pre-clinical drug development.