

# Population based *in vivo* genetic models of tumor metastasis

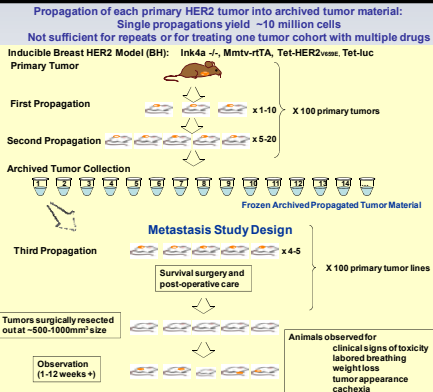
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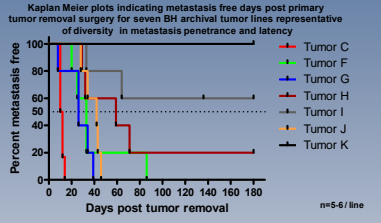
## Abstract

Metastatic disease is one of the major challenges in cancer therapeutics. Despite its importance, the molecular determinants underlying metastasis are poorly understood, primarily due to the paucity of relevant preclinical models. Existing preclinical models are mostly based on serial selection of distant metastases following xenograft implantation of human tumor cell lines in mice, an inefficient process which may reveal little about the biological changes driving spontaneous, visceral metastasis. Thus we have defined a need for genetically defined, reproducible tumor metastasis models to allow for identification of the genetic pathways regulating metastasis and for preclinical evaluation of drug response *in vivo*. To this end, we are utilizing a murine, *Ink4a*<sup>-/-</sup>, inducible HER2-driven breast cancer model (BH) to identify and characterize tumors capable of metastasis. Previously we described an archive of relevant preclinical models. Existing preclinical models are mostly based on serial selection of distant metastases following xenograft implantation of human tumor cell lines in mice, an inefficient process which may reveal little about the biological changes driving spontaneous, visceral metastasis. Thus we have defined a need for genetically defined, reproducible tumor metastasis models to allow for identification of the genetic pathways regulating metastasis and for preclinical evaluation of drug response *in vivo*. Following an initial subcutaneous implantation, allograft tumors derived from each archival tumor line were surgically resected. Mice were monitored for up to 180 days post-surgery for the appearance of distal subcutaneous tumors or clinical signs of distant visceral metastasis. To date, more than 47 distinct tumor lines have been characterized for their metastatic potential by penetrance, latency and preferred sites of metastasis including lung, pericardium, liver, ovary, spleen, lymph nodes, kidney and other subcutaneous sites (25 mice / line). Observed differences in these parameters appear to be governed by the underlying properties of each tumor line. Global gene expression profiles for a subset of the primary excised tumor and their resultant distal metastases revealed divergent gene expression patterns acquired in metastases. This platform, combining extensive phenotypic characterization of a panel of related, but genetically distinct tumors and their metastases, with the comprehensive molecular profiling, affords a unique pre-clinical opportunity for identifying the subtle biological changes that propel some tumors to establish distant metastatic sites while allowing others to remain relatively dormant, ultimately leading to more effective therapeutic strategies against metastatic disease.

## Generation of propagated breast HER2 tumor archive

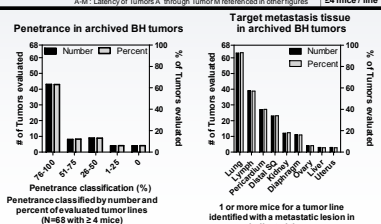
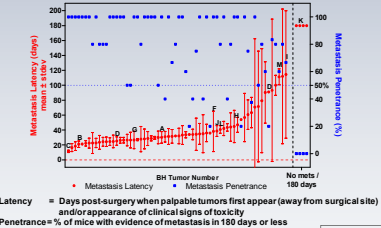


## BH archival tumors show diversity in latency and penetrance of metastatic phenotype



Tumors C, G and J were highly metastatic, with 100% penetrance in under 50 days, while Tumor F was 80% penetrant in under 50 days. Tumor K had no metastases within 180 days of tumor removal. Tumors I and J are representative of tumors with partial penetrance, either low or high, respectively.

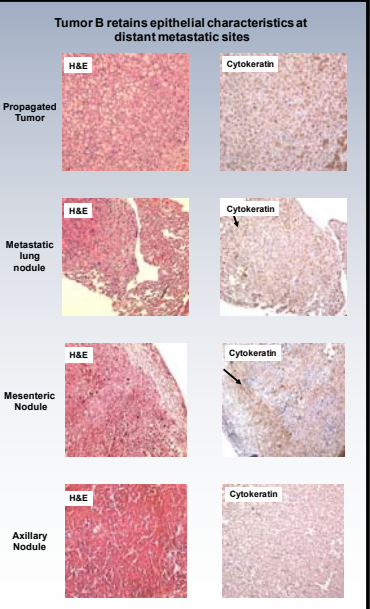
## Metastasis and penetrance of 68 archived BH tumors



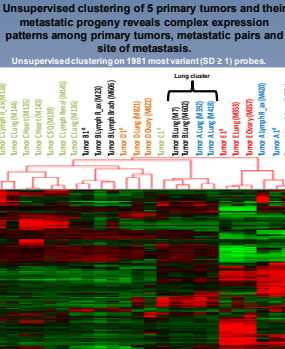
## Metastases present in a variety of tissues



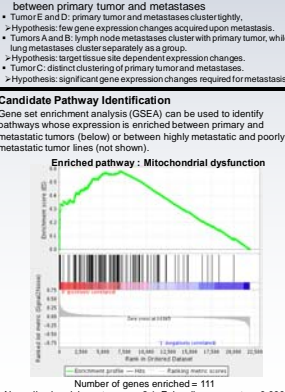
## Histological characteristics of metastatic nodules



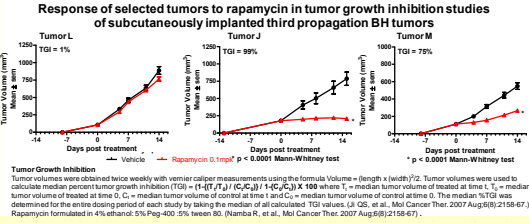
## Gene expression changes between primary tumors and metastases



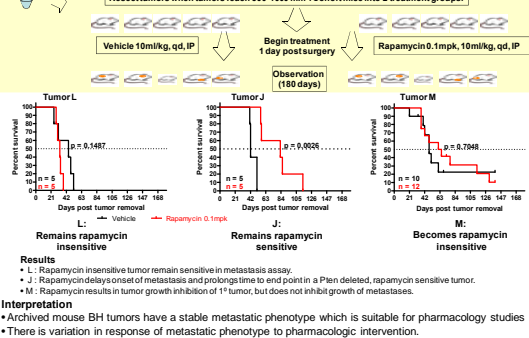
## Candidate Pathway Identification



## Metastasis models with archived BH tumors are suitable for pharmacology studies



## For selected tumors, a cohort of mice with third propagation BH tumors were evaluated for the effects of rapamycin treatment on the metastatic phenotype.



## Summary

- To date, 43 (63%) of AVEO's BH tumor lines metastasize with a penetrance of 76-100%. 8 (12%) of tumor lines demonstrate little to no metastasis (0-25% penetrance).
- Tumor lines predominantly metastasize to lung & lymph tissue.
- The metastatic phenotype is robust, reproducible and suitable for pharmacology studies.
- The archived tumors display diversity in response to therapeutic intervention in both subcutaneous and metastatic tumor growth assays.