Tepotinib treatment inhibits tumor growth and affects tumor permeability in an intracranial PDX model of *MET*-amplified NSCLC brain metastasis

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Disclosure Information

Joachim Albers

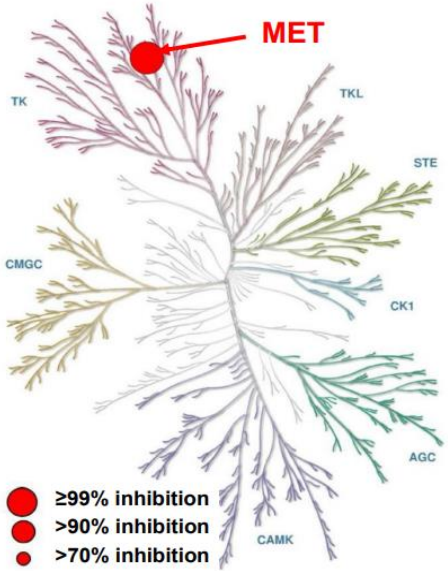
I have the following relevant financial relationships to disclose:

- Employee of the healthcare business of Merck KGaA, Darmstadt, Germany
- Stockholder in Merck KGaA, Darmstadt Germany

Tepotinib

- Highly selective, ATP-competitive, reversible, potent MET TKI
  - At 1 µM, only MET is inhibited >70% out of a panel of more than 300 kinases
  - Single digit nanomolar IC_{50} in MET driven cancer cell lines
- Potent anti-tumor activity in preclinical in vivo models with various mechanisms of oncogenic MET activation
- Preclinical brain penetration demonstrated (Wistar rats)
  - $K_{p\ u,u}$ (ratio of free tepotinib brain vs plasma concentration) was 0.25, i.e. 25% of free tepotinib levels in brain, relative to levels found in plasma
- Global approval for the treatment of patients with *MET* exon 14 skipping NSCLC
- Efficacy reported in NSCLC with *MET* amplification, both as primary driver and as mechanism of resistance

Kinome showing inhibition of 305 kinases (Eurofins) at 1 µM of tepotinib



1 µM corresponds to approximately 20-fold of the free maximal concentration (C_{max}) at the clinical dose of 500 mg, qd

IC_{50}, half-maximal inhibitory concentration; MET, mesenchymal-epithelial transition; NSCLC, non-small cell lung cancer; qd, every day; TKI, tyrosine kinase inhibitor.; ~~NCCN, National Comprehensive Cancer Network~~

Identification of MET dependent patient-derived NSCLC brain metastasis models

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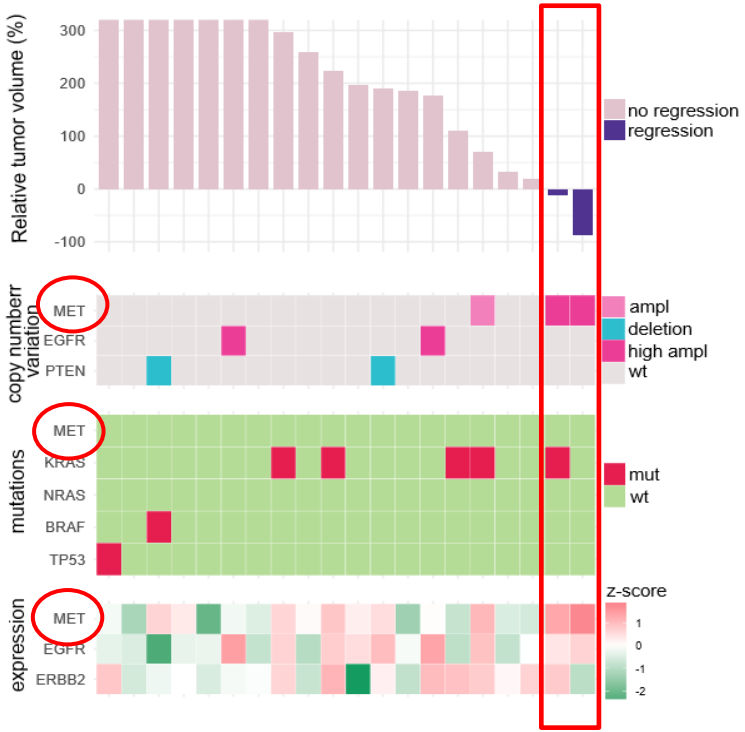
- Brain metastases are particularly common in patients with NSCLC
- We performed an in vivo screen of tepotinib in 20 subcutaneous xenografts derived from human NSCLC brain metastasis
- Tumor regression was observed in 2/20 models
- Molecular profiling revealed that the 2 most sensitive models (LU5349 and LU5406) have high-level *MET* amplification (GCN>10)
- In a previous study, tepotinib treatment led to regression of established intracranial tumors from these 2 models



Friese-Hamim et al. *Lung Cancer.* 2022

GCN, gene copy number; NSCLC, non-small cell lung cancer.

Study objectives

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1)

How does tepotinib distribute in the brain and brain tumors?

2)

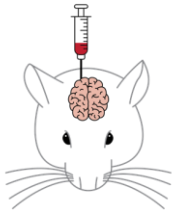
How does exposure of tepotinib in the brain tumor correlate with PD biomarker modulation and surrogate markers of anti-tumor efficacy?

3)

Does tepotinib affect vascular permeability?

Study overview

Intracranial inoculation

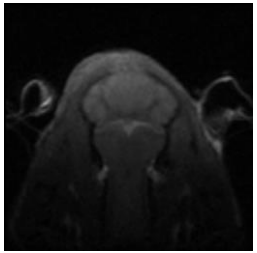


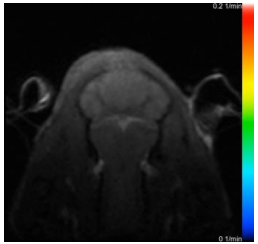
200 mice/model injected
→ **90 enrolled in study**

Treatment	N/imaging and tissue collection timepoints*		
	Day 0	**Day 6** (LU5349) **Day 2** (LU5406)	**Day 16** (LU5349) **Day 9** (LU5406)
Vehicle p.o.	10	10	10
Tepotinib p.o. (125 mg/kg)	10	10	10
Tepotinib p.o. (300 mg/kg)	10	10	10

*Tissue collection 2 hours after tepotinib treatment.

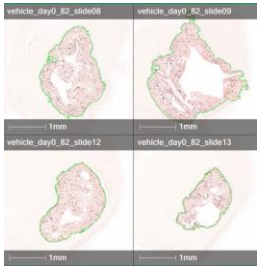
T2-weighted MRI
(tumor volume)

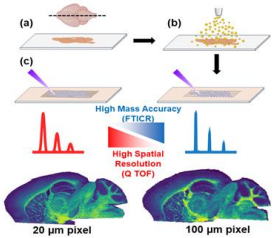




DCE MRI (K^{trans})

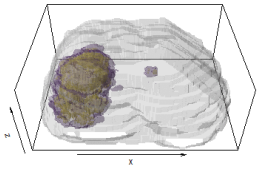
IHC





MALDI-MSI

Data integration



3D reconstruction

p.o., per os = oral treatment; DCE, dynamic contrast enhanced; IHC, immunohistochemistry; MALDI MSI, matrix assisted laser desorption ionization mass spectrometry imaging; MRI, magnetic resonance imaging.

Tepotinib treatment resulted in regression of both intracranial *MET*amp PDX tumor models

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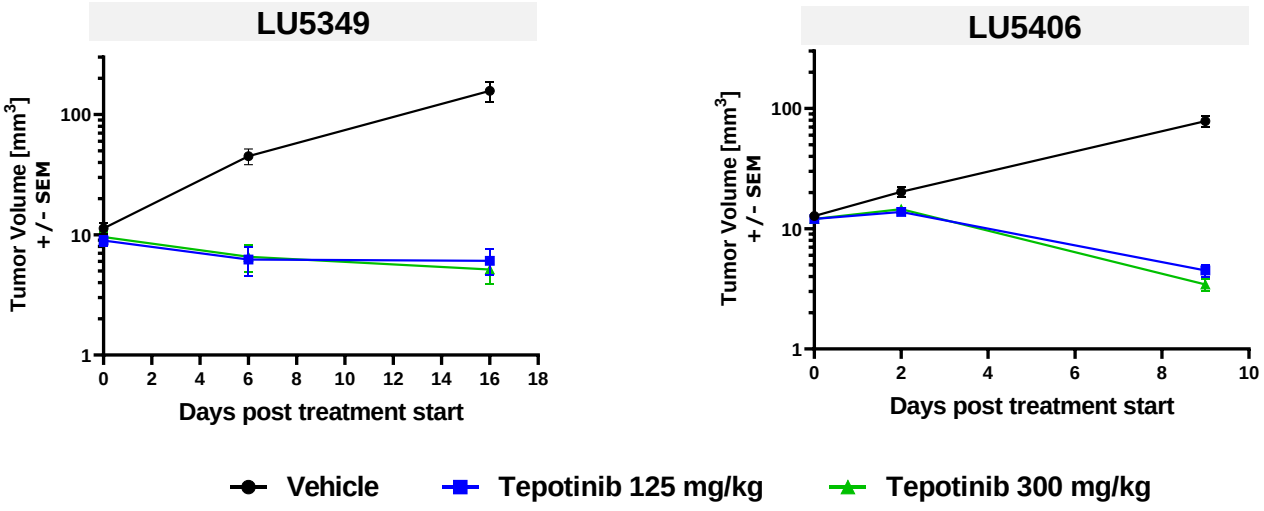
- T2-weighted MRI imaging revealed regression upon tepotinib treatment
- No difference in tumor volume between clinically relevant and higher dose of tepotinib



*MET*amp, *MET* amplifications; MRI, Magnetic Resonance Imaging; PDX, patient-derived xenograft.

MALDI-MSI analysis of intratumoral tepotinib concentrations

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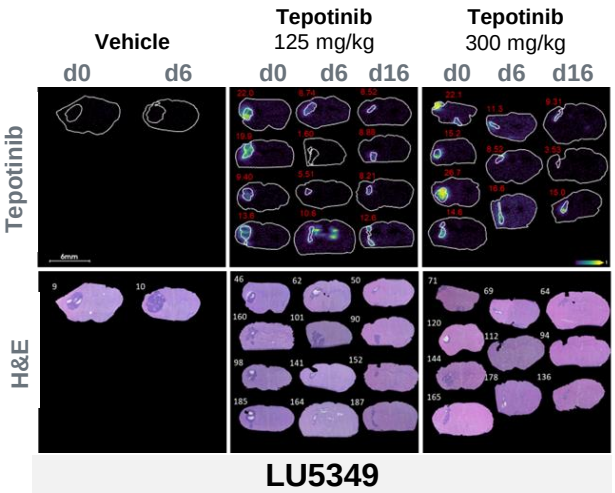
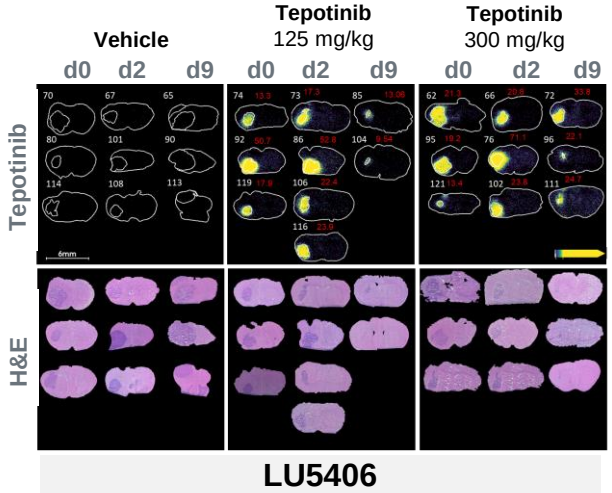
- MALDI-MSI was performed to map, with quantitative precision, the spatial distribution of tepotinib in brain tumors and normal brain tissue
- Mice were euthanized 2 hours after tepotinib treatment and brains collected for MALDI-MSI
- Accumulation of tepotinib in tumors compared to normal brain





H&E, hematoxylin & eosin; MALDI MSI, matrix assisted laser desorption ionization mass spectrometry imaging.

Tepotinib concentrations are higher in brain tumors than in normal brain tissue

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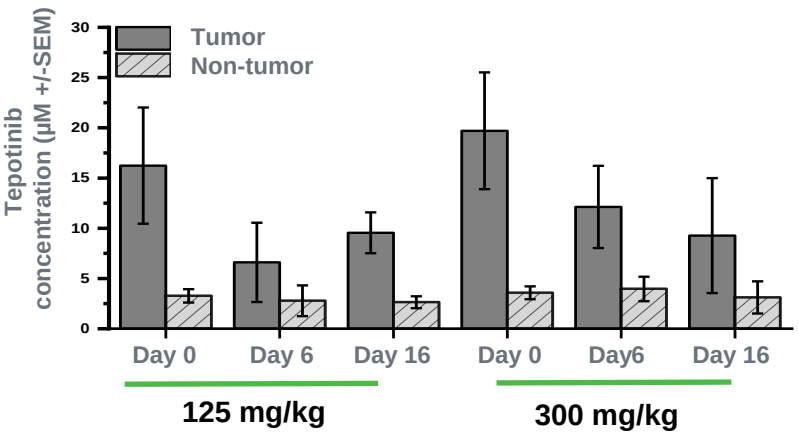
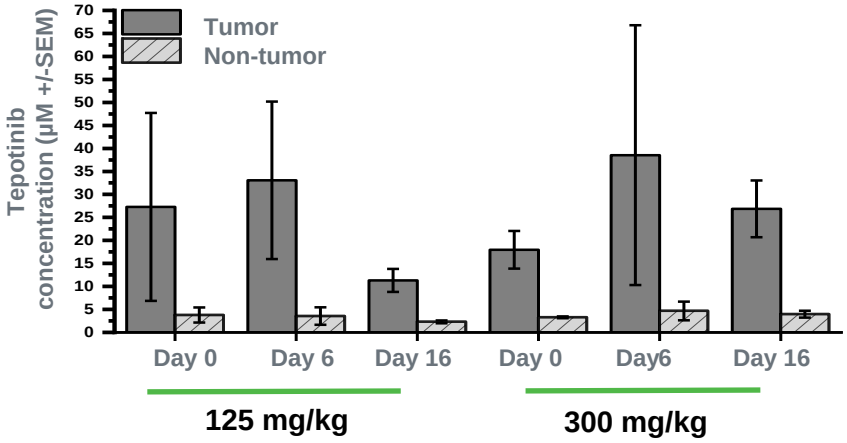
- Quantification of MALDI-MSI data demonstrates that tepotinib was enriched in tumor versus normal brain tissue
- No proportional increase in brain tumor and normal brain concentrations with higher dose
- Concentrations in normal brain tissue remain stable over time, suggesting that tepotinib does not accumulate in normal brain and does not compromise the BBB



LU5349



LU5406

BBB, blood-brain barrier; MALDI MSI, matrix assisted laser desorption ionization mass spectrometry imaging; SEM, Standard error of the mean

Tepotinib treatment results in inhibition of MET autophosphorylation in PDX model LU5349

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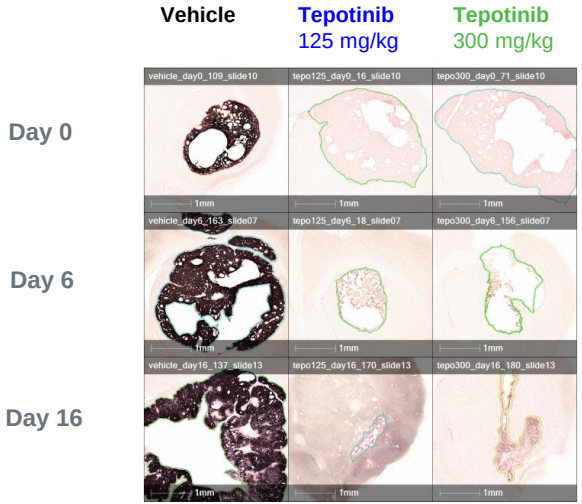
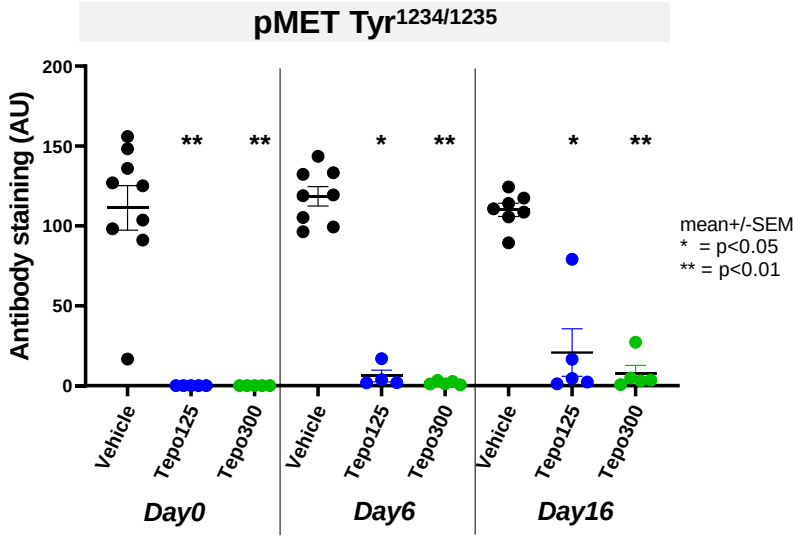
- MET autophosphorylation is an established preclinical and clinical PD biomarker for MET inhibitors
- pMET$^{Tyr1234/1235}$ is significantly decreased in brain metastasis after tepotinib treatment
- No significant difference between the two dose levels





Brains were collected 2 hours after administration of tepotinib at the indicated timepoints

Kruskal-Wallis test (compared treatment groups to respective vehicle group)

MET, mesenchymal-epithelial transition; PD, pharmacodynamic; Tepo, Tepotinib; SEM, Standard error of the mean

Tepotinib treatment results in significant inhibition of tumor cell proliferation in PDX model LU5349

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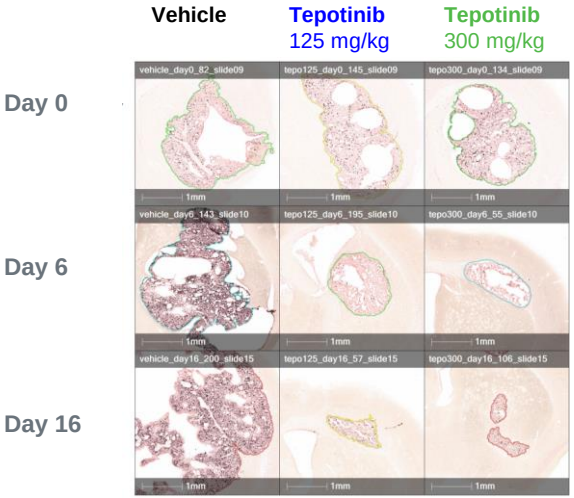
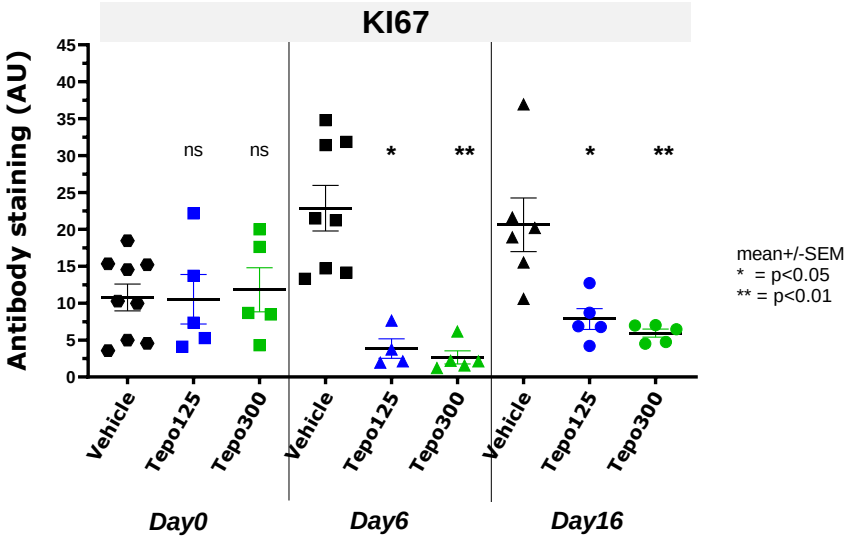
- Tepotinib treatment results in significant reduction of KI67 staining in tumor cells, indicating inhibition of cell proliferation
- No significant difference between the two dose levels



Brains were collected 2 hours after administration of tepotinib at the indicated timepoints



Kruskal-Wallis test (compared treatment groups to respective vehicle group)

MET, mesenchymal-epithelial transition; Tepo, Tepotinib; SEM, Standard error of the mean

Tepotinib treatment decreases vascular permeability in NSCLC brain metastasis PDX model LU5349

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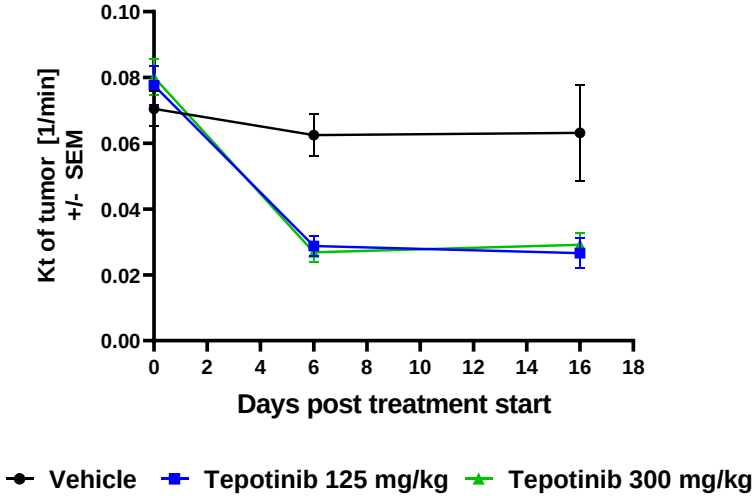
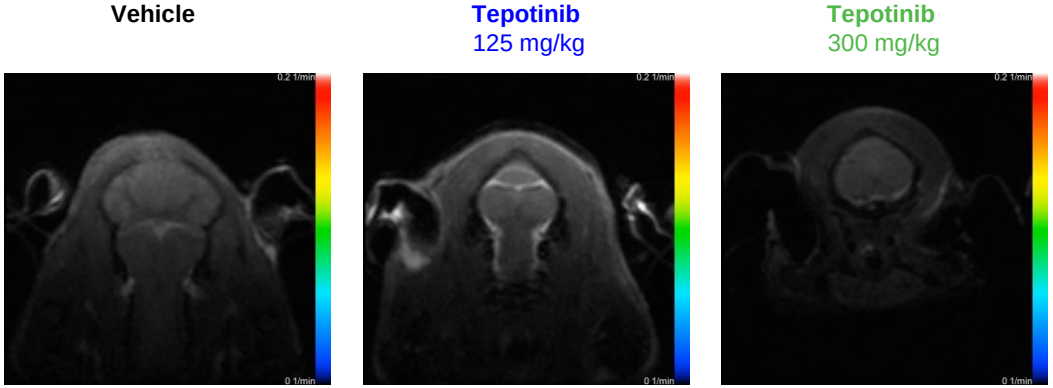
- Tepotinib treatment induces a sharp decrease in K^{trans}, indicative of a decrease in vascular permeability
- Vascular permeability is heterogenous in the tumor





Transfer constant (K^{trans}) values over Time (by DCE MRI) – fly through for representative mouse in each group on **Day 16**

DCE, dynamic contrast enhanced; MRI, magnetic resonance imaging; NSCLC, non-small cell lung cancer; PDX, patient-derived xenograft; SEM, Standard error of the mean; Kt, transfer constant

Tepotinib treatment decreases vascular permeability in NSCLC brain mets PDX model LU5406

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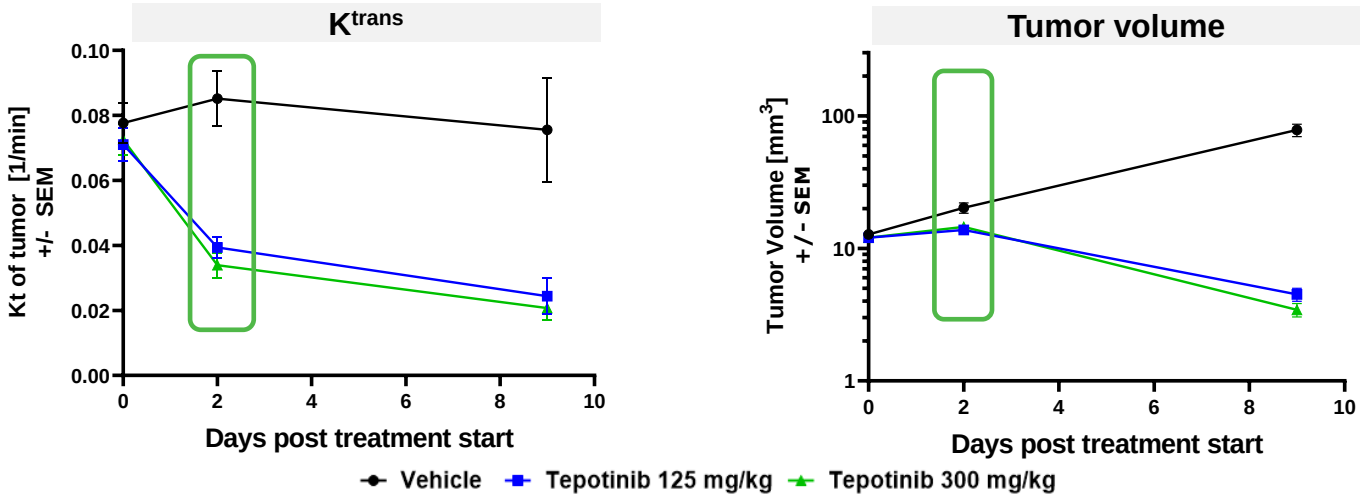
- Tepotinib treatment induces a sharp decrease in K^{trans} , indicative of a decrease in vascular permeability – similar level of intratumoral heterogeneity as in model LU5349
- Decrease in K^{trans} precedes decrease in tumor volume
- K^{trans} could be a potential predictive biomarker for intracranial tepotinib response



BM, brain metastases; PDX, patient-derived xenograft; SEM, Standard error of the mean; Kt, transfer constant

Extravasation of IgG is decreased upon tepotinib treatment (LU5349)

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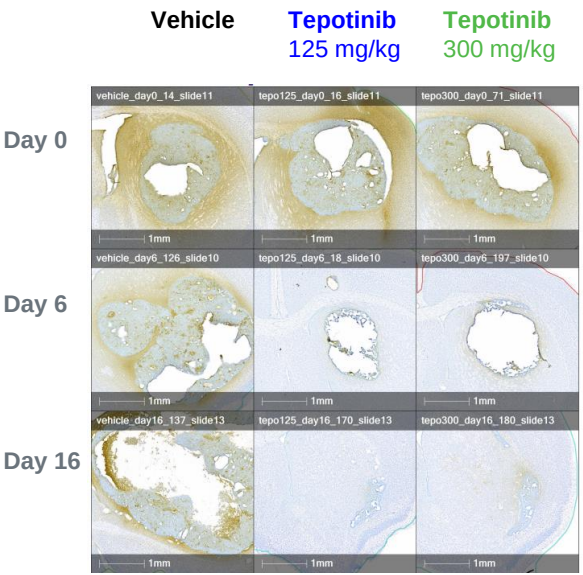
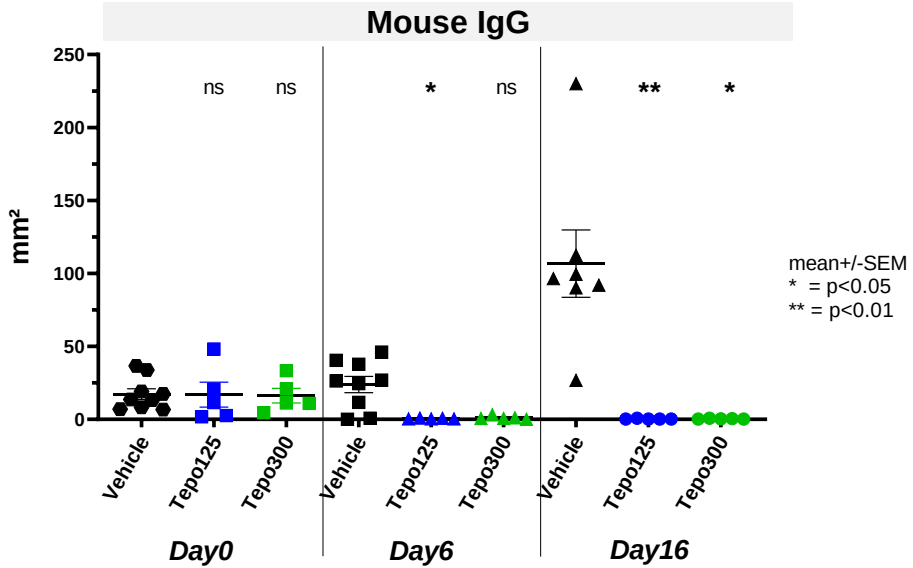
- Extravasation of IgG is a marker of BBB disruption and can be analyzed via IHC
- Tepotinib treatment results in decreased IgG immunostaining in the tumor and in normal tissue adjacent to the tumor, suggesting normalization of the BBB and BTB





Kruskal-Wallis test (compared treatment groups to respective vehicle group)

BBB, blood-brain barrier; BTB, blood-tumor barrier; IgG, immunoglobulin G; IHC, immunohistochemistry; SEM, Standard error of the mean

Conclusions

- Tepotinib accumulates in *MET*amp NSCLC brain metastasis PDX tumors and has pronounced anti-tumor activity
- A tepotinib dose higher than the clinically relevant dose did not result in an increased intratumoral exposure, likely due to limited absorption of the drug at the higher dose
- Tepotinib treatment decreases vascular permeability (K^{trans}) and IgG immunostaining, suggesting BBB and BTB normalization
- Tepotinib exposure remains at therapeutic levels and achieves durable tumor control despite a decrease in vascular permeability, potentially a consequence of the BBB-penetrating properties of the drug
- DCE MRI may serve as a predictive biomarker of intracranial tumor response to tepotinib in the clinic, however this needs to be validated in a clinical study
- In patients with *MET* exon 14 skipping, tepotinib demonstrated an intracranial objective response rate of 66.7%, indicating that findings from our study translate to the clinic

BBB, blood-brain barrier; BTB, blood-tumor barrier; DCE, dynamic contrast enhanced; IHC, immunohistochemistry; MET, mesenchymal-epithelial transition; *MET*amp, *MET* amplifications; MRI, magnetic resonance imaging; NSCLC, non-small cell lung cancer; PDX, patient-derived xenograft.

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Outlook

- Co-registration of MRI with MALDI-MSI and/or IHC data
- Correlate data in individual animals with high versus low tepotinib exposure
- Spatially resolved metabolomics