Residence time of DO-2, a novel deuterated MET kinase inhibitor on the endogenous target: Differentiates DO-2 from competitor agents.

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Poster 407

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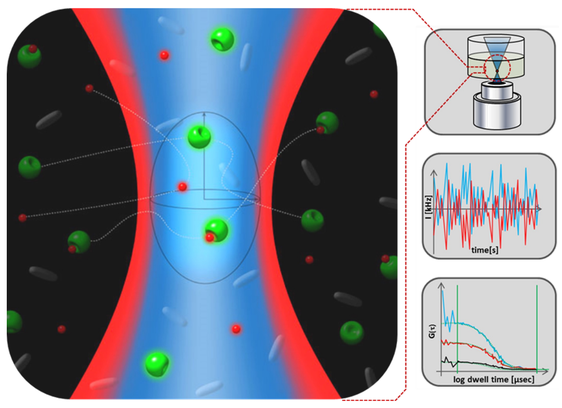
Introduction

- DO-2 is a highly selective, deuterated MET kinase inhibitor that is currently undergoing clinical evaluation (NCT NCT05752552). Overexpression of wild type (wt) receptor due to amplification, exon 14 skipping mutation or fusions are known to be oncogenic due to continuous pathway overactivation. It is known that wtMET plays critical physiological roles including in endothelial cells that control vascular tone, that when continuously inhibited could result in unwanted 'on-target' toxicities.
- We have employed Fluorescence Cross Correlation Spectroscopy (FCCS) as a versatile platform technology to interrogate the molecular interactions under physiological conditions. The approach has been successfully applied for membrane proteins to yield equilibrium binding constants and kinetic parameters. FCCS analysis carried out in crude cell lysates provides a precise and complex read out including exact concentrations, molecular size binding state, complex half-life and allows for differentiation between on and off target binding.
- Target Occupancy quantification of the endogenous MET demonstrates target engagement by DO-2 in the relevant cancer models. This method is of critical importance to optimize dosing and therapeutic regimen for drug development.

FCCS Technology



Single molecule detection in 4 to 20 µl sample volumes

Recording of diffusion-events

Fitting of data to obtain concentration and diffusion time

Tools used

Solubilised MET-GFP

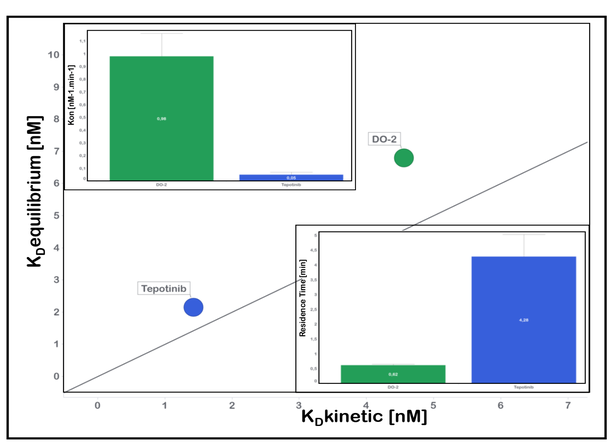
Tracer binding competitive to the MET kinase pocket

Solubilised untagged MET

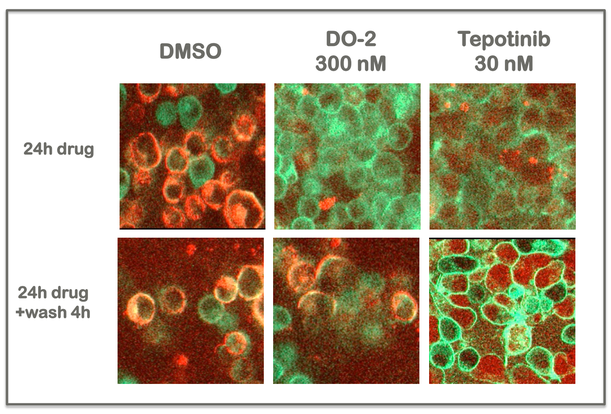
Labelled antibody binding to MET not interfering with drug binding

Tested drugs: DO-2 and tepotinib

MET-GFP Results

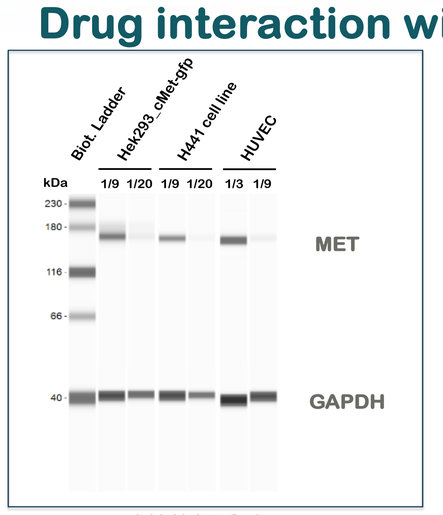


- Using solubilized MET-GFP expressed in HEK293 cells the equilibrium and kinetic interaction data of drugs have been determined using FCCS
- Overall DO-2 has a 3 fold lower Ki compared to Tepotinib.
- DO-2 binds faster to MET-GFP, but have a shorter residence time on the target



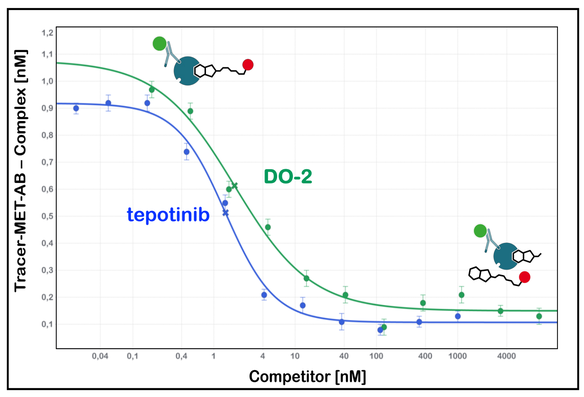
- Live cell imaging using a stable HEK293 cell line expressing MET-GFP confirmed competitive binding of the tracer and shorter half-life of the DO-2 interaction to the target in living cells.
- Snapshots of a confocal time laps video 7 minutes after addition of tracer– (representative images)

Drug interaction with endogenous MET in lysates

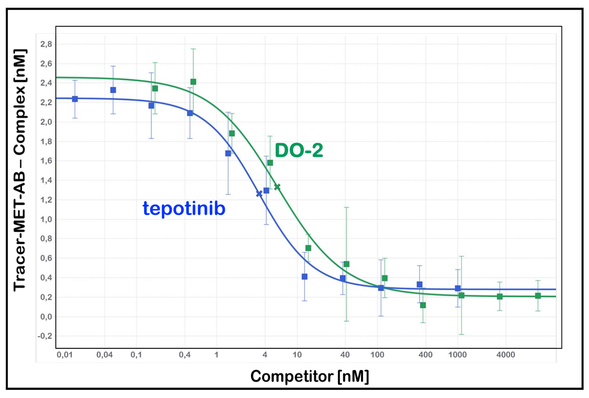


- HUVEC cells and H441 cells were selected to monitor drug binding to endogenous MET.
- HUVEC cell express approximately 1/3 of MET compared to H441 as determined by western blot
- FCCS data confirmed the difference in MET levels in the cell lines using a labelled antibody and the tracer to detect and quantify endogenous MET in the lysate.
- Using this assay, the activities of DO-2 and tepotinib on the endogenous unmodified MET were confirmed using two relevant cellular models (normal and cancer)
- Absolute binding activities are related to the expression levels of the target

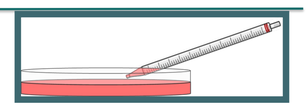
HUVEC Lysate



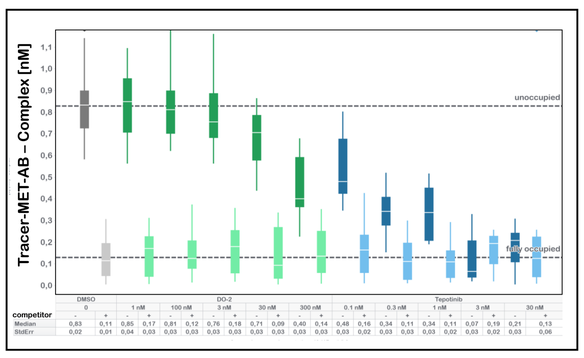
H441 Lysate



Target Occupancy in living cells

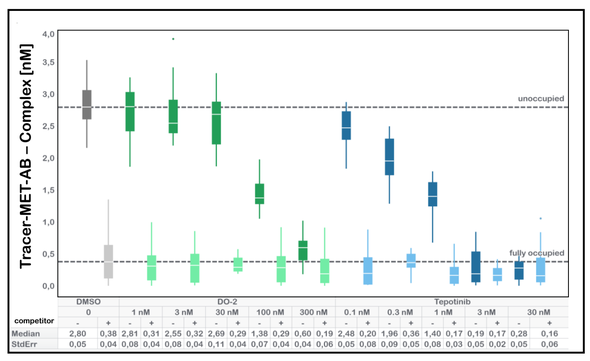


HUVEC

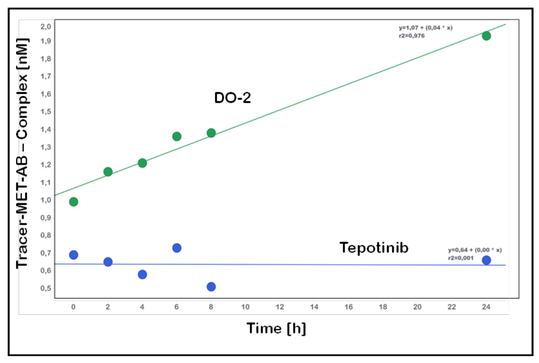


- HUVEC cells and H441 treated with different doses of inhibitors for 8h.
- Cells were harvested and the occupancy of the target was determined by FCCS.
- Sample specific background levels were determined by the addition of high concentration of a competitor.
- For the Chase experiment H441 cells were treated for 8h, washed and sample were taken at the times indicated.

H441 - Pulse



H441 - Chase



Conclusions and Clinical Consequences

- DO-2 and tepotinib found to have binding affinities of 6.18 nM and 2.15 nM against endogenous MET.
- DO-2 has 'fast on/fast off' binding kinetics, with continued target engagement being dependent on continuous drug exposure.
- Tepotinib has 'slow on/slow off' binding kinetics with extended residence time >24 hrs with target engagement being independent of continuous drug exposure.
- Unlike many other oncogenic mutations, the basis of MET driven disease is the over activation of the wild type MET receptor. MET also plays key roles in many physiological functions including the control of vascular tone by endothelial cells.
- Effective and tolerable therapeutics need to have balanced 'on target' effects on tumour cells whilst minimising 'on target' effects on normal cells that also use MET signaling as part of normal physiology.
- The different binding kinetics determined for DO-2 vs tepotinib may underlie the lack of peripheral edema seen with DO-2 compared to the high levels (~67%) reported with tepotinib*

* Mazieres et al. Tepotinib Treatment in Patients With MET Exon 14-Skipping Non-Small Cell Lung Cancer: Long-term Follow-up of the VISION Phase 2 Nonrandomized Clinical Trial. JAMA Oncol. 2023 Sep 1;9(9):1260-1266. doi: 10.1001/jamaoncol.2023.1962.

Disclosures:
Timothy Perera is the founder and CEO of DeuterOncology and is a share and stock option holder
Frank Becker is founder and CEO of Intana Bioscience GmbH

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