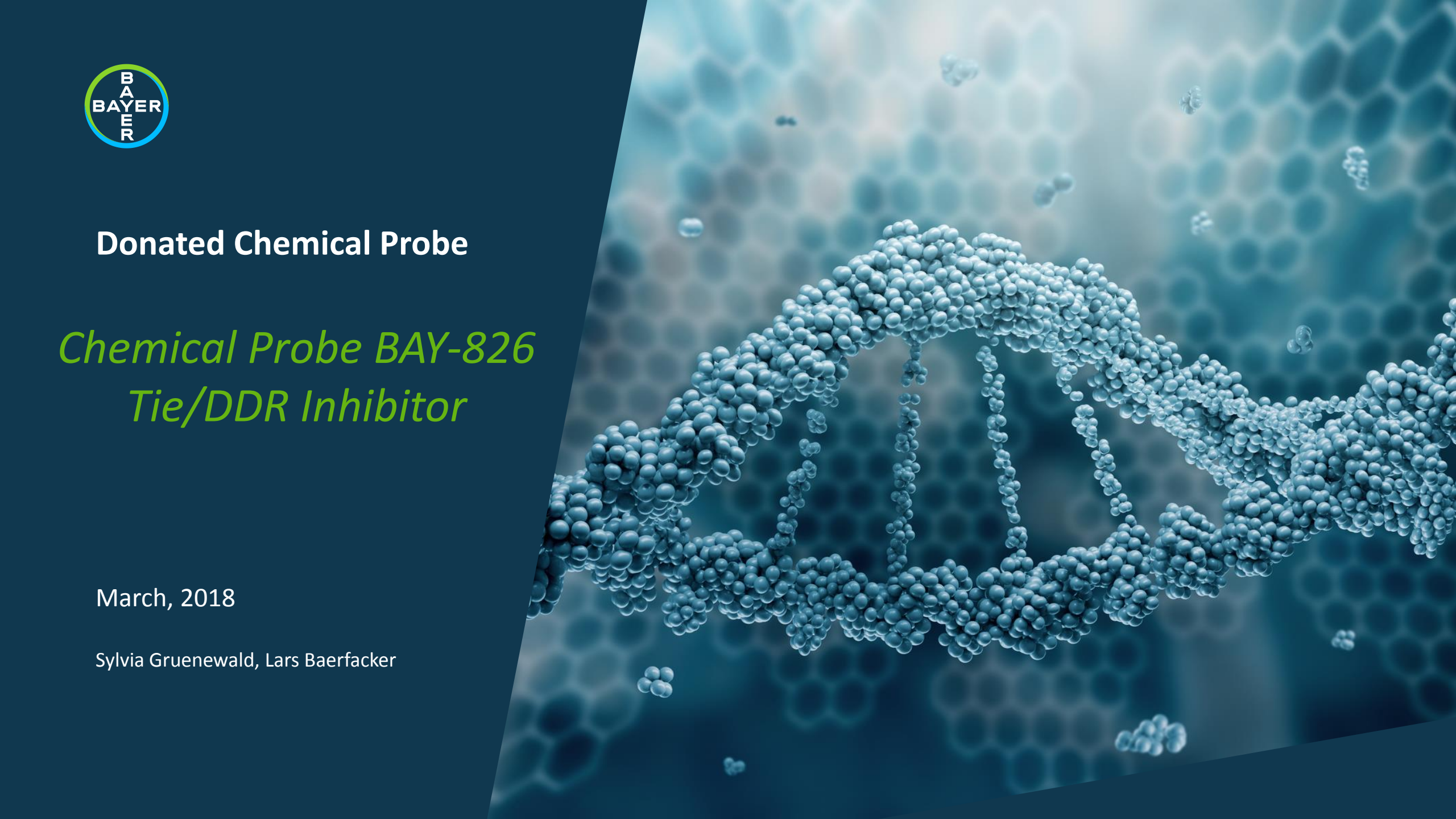


Donated Chemical Probe

Chemical Probe BAY-826
Tie/DDR Inhibitor

March, 2018

Sylvia Gruenewald, Lars Baerfacker

Tie/DDR probe BAY-826:

Scientific rationale: Tie2 as an anti-cancer target

RTK Tie2 as a "classical" anti-angiogenesis target

// Ang1/2-Tie2 signaling: important role in angiogenesis and vessel maturation

// Tie2 inhibition: impairs angiogenesis & reduces tumor growth

shows combination benefit with anti-VEGFR therapy

Tie2 as anti-tumor cell target beyond angiogenesis

// Survival of **Tie2-positive AML cells** sustained through autocrine Ang1/Tie2 loop

// **Tie2-positive hematological tumor cells** may adhere to the bone marrow niche thus being protected from chemotherapy

// Activation of **gliomal Tie2** increases tumorigenesis and invasive phenotype *in vivo*

// **Tie2** activation **in gliomal and brain tumor stem cells** contributes to chemoresistance

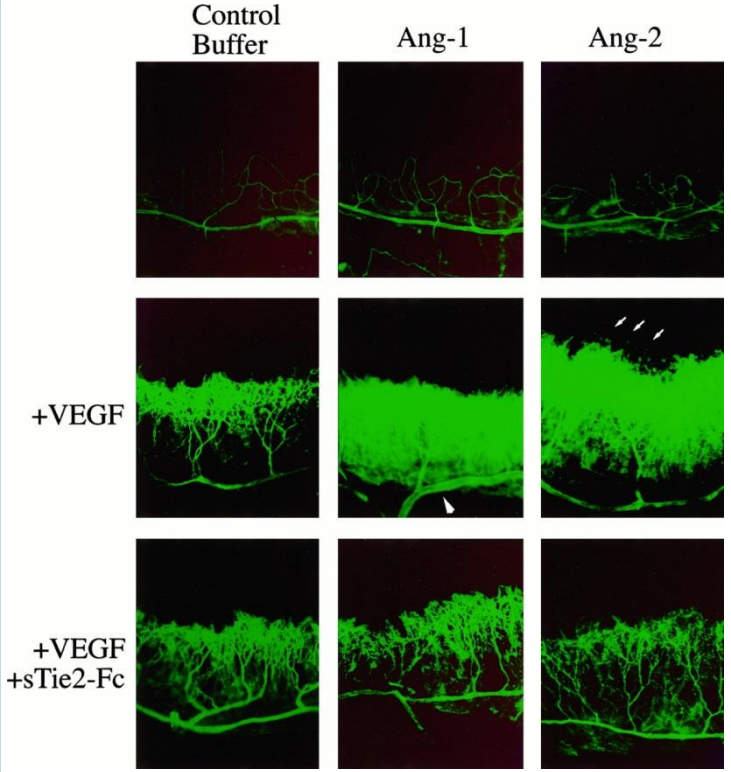
// Infiltration of **Tie2-expressing macrophages** (TEMs) is implicated to promote angiogenesis and **metastatic dissemination**, potentially of relevance in various tumor indications incl. HCC.

Tie/DDR probe BAY-826:

Disease hypothesis based on literature data

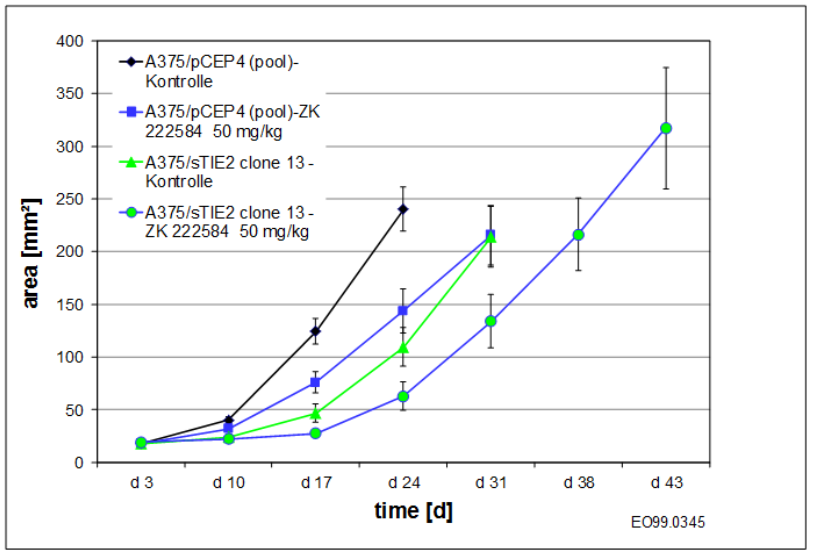
Angiogenesis - Tie2 function is essential for development of embryonic vasculature, for maintenance of blood vessels and for the maturation of newly formed vessels

In situ BS-1 lectin fluorescent staining of corneal limbus vessels 6 days after pellet insertion



Asahara, T. *et al.* (1998) Circ Res 83, 233-240

Comparison of the sTie2 secreting A375 melanoma xenografts with parental cells with and without treatment of PTK787/ZK222584



Siemeister *et al.* (1999) Canc. Res. 59, 3185 and WO2001/097850

// sTie2-Fc precluded modulation of VEGF-induced neovascularization by either angiopoietin (Ang2 as well as Ang1) *(left)*

// Tie2 inhibition on top of VEGFR2 inhibition improves efficacy

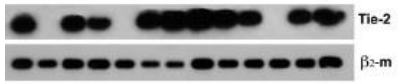
Tie/DDR probe BAY-826:

Disease hypothesis based on literature data

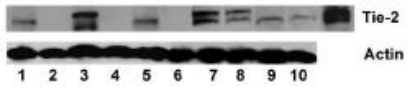
AML - Tie2 & Ang1 expressed in subset of AML blasts

Tie2

RT-PCR[1)]



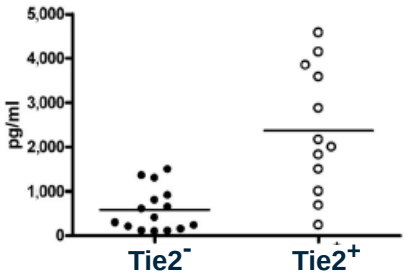
Western Blot



1) No info on patient number of RT-PCR samples

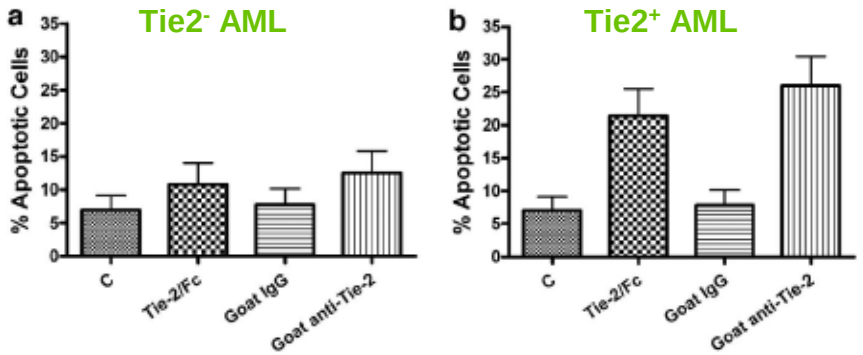
Ang1

ELISA[2)]



2) 5d culture supernatants from leukemic blasts

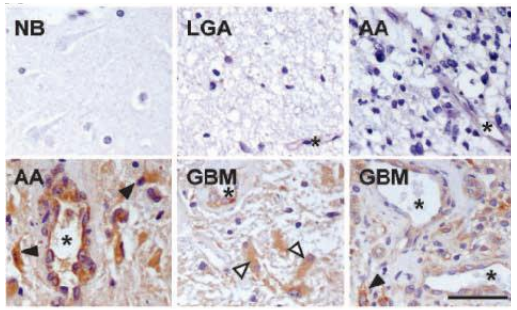
Blocking of Ang1-Tie2 signaling in Tie2+ but not Tie2- AML cells increases apoptosis



a) Riccioni *et al*. (2007) *Stem Cells,* 25, 1862. b) Arai *et al.* (2004) *Cell,* 118, 149. c) Ichihara *et al*. (2011) *BBRC*, 416, 239.

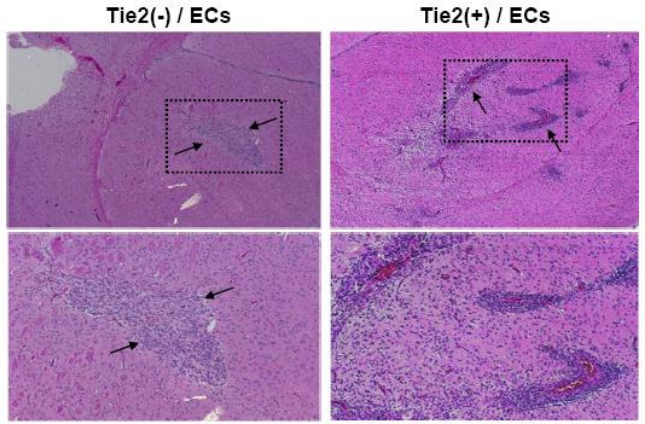
Glioma - Tie2 expression in neoplastic glial cells correlates with grade

Tie2 staining on human TMA sections
normal brain (NB), low grade astrocytoma (LGA), anaplastic astrocytoma (AA), and glioblastoma multiforme (GBM)



Activation of gliomal Tie2 increases tumorigenesis and invasive phenotype *in vivo*

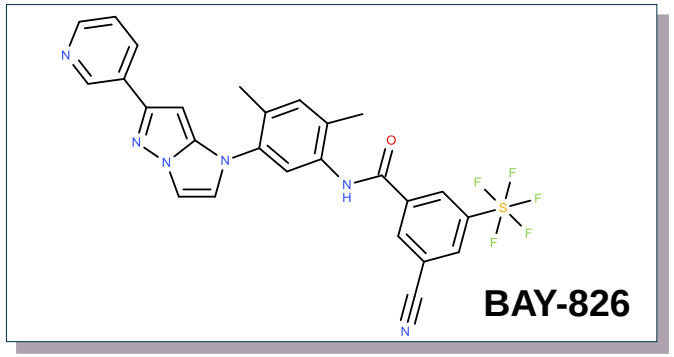
U251.vector or U251.Tie2 cells were injected with ECs into the brains of immuno-compromised mice ➔ infiltrative/multi-focal component of tumors in Tie2+/EC



a) Lee *et al*. (2006) *Mol. Canc. Res.,* 4, 915. b) Liu *et al*. (2010) *Oncotarget*, 1, 700. c) Martin *et al*. (2009) *Oncogene*, 28, 2358.

Tie/DDR probe BAY-826:

Overall profile



▪ **Molecular Properties**

MW [g/mol]	559
MWcorr [g/mol]	490
TPSA [Å2]	88
Rotatable bonds	5

▪ **PhysChem**

Sw $^{pH\ 6.5}$ [mg/L]	1.8
log D (pH 7.5)	3.61

▪ **Pharmacology**

Tie2 K_D (KINOMEscan™)	1.6 nM
Tie2 IC_{50} (in-house kinase assay)	0.45 nM
Tie2 IC_{50} (HUVEC pTie2-ELISA)	1.3 nM
Tie1 K_D (KINOMEscan™)	0.9 nM
DDR1 K_D (KINOMEscan™)	0.4 nM
DDR2 K_D (KINOMEscan™)	1.3 nM
VEGFR2 K_D (KINOMEscan™)	1.6 µM
VEGFR3 IC_{50} (in-house kinase assay)	0.44 µM
FGFR1/3 IC_{50} (in-house kinase assay)	>10 µM
PDGFR-β IC_{50} (in-house kinase assay)	>20 µM

▪ **In vitro PK**

		Clint [L/h/kg]	Fmax [%]
LM	Human	0.43	68
	Mice		
	Rat	1.91	54
Hep	Rat	0.96	77

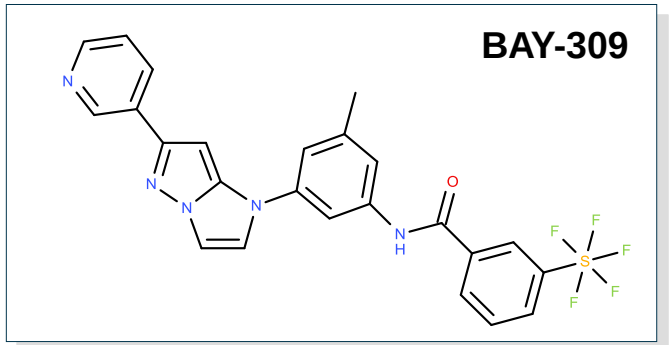
CaCo2	A-B [nm/s]	B-A [nm/s]	Ratio
	78	55	0.7

▪ **Safety (LeadProfilingScreen, total # of assays:68)**

BAY-826 (10 µM)	Adenosine A3 (65 % inh.), Opiate κ (75% inh.), hERG (66 % inh.), Sodium Channel site2 (84% inh.)

Tie/DDR probe BAY-826:

Negative control BAY-309



- **Molecular Properties**

MW [g/mol]	519
MWcorr [g/mol]	450
TPSA [Å2]	64
Rotatable bonds	5

- **PhysChem**

$Sw^{pH\ 6.5}$ [mg/L]	0.14
log D (pH 7.5)	3.71

- **Pharmacology**

Tie2 K_D	>20 µM

- **In vitro PK**

CaCo2	A-B [nm/s]	B-A [nm/s]	Ratio
	No data (below detection limit)		

Tie/DDR probe BAY-826:

Biochemical and cellular potency and selectivity

Biochemical activity[a]	K_D [nM]
Tie2	**1.6 (0.5, IC_{50})**
Tie1	**0.9**
DDR1	**0.4**
DDR2	**1.3**
LOK	**5.9**
EPHB6	25
LYN	40
MERTK	66 (IC_{50})
ABL1-non-phosphorylated	38
ABL1-phosphorylated	510
ABL1 (T315I)-non-phosphorylated	100
ABL1 (T315I)-phosphorylated	310
KIT	150 (>20000, IC_{50})
BRAF (V600E)	370
BRAF	730
RAF1	1400
VEGFR2	1600 (118, IC_{50})
VEGFR3	439 (IC_{50})
FGFR1	16200 (IC_{50})
FGFR3	12800 (IC_{50})
PDGFR-β	>20000 (IC_{50})

[a] K_D values determined by DiscoverX Corp. on the KINOMEscan platform

Summary of cellular NanoBRET™ assay data @SGC

	BAY-826 IC50 (normed to Tie-2 data)*	Ratio of IC50s (BAY-309/BAY-826)*
Tie2	1.0	1604.7
DDR2	4.5	3056.1
DDR1	1.4	369.9
Tie1	8.1	23.4
STK10* **	317.0	
EPHB6	5822.8	2.9

* Dose response curves and derived IC_{50} values, see backup section

// BAY-826 binds to Tie1, Tie2, DDR1 and DDR2 with a K_D of ~ 1 nM

// BAY-826 is selective versus the known angiogenic RTKs VEGFR1/2/3, FGFR1/2/3/4 and PDGFR-α/β

// BAY-826 is equipotent vs DDR1/2 (and Tie1 kinase) in cellular assays and more than three orders of magnitude less active vs STK10 and EPHB6 kinase

// The neg. control BAY-309 reveals a sufficient cellular selectivity vs BAY-826 for all six kinases tested

Tie/DDR probe BAY-826:

Summary / conclusion

Probe criteria	
Inhibitor/agonist potency: goal is < 100 nM (IC50, Kd)	**Surpasses criteria**; biochemical assay (Tie-2) with IC_{50} 0.45 nM;
Selectivity within target family: goal is > 30-fold	Surpasses criteria; selectivity >100 fold vs all other angiogenic kinases, (# of kinases tested 453) albeit cellular equipotency vs DDR1/2 and Tie-1
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	**Surpasses criteria**; Promising LeadProfilingScreen: 4 out of 68 assays hit (all in µM potency range)
On target cell activity for cell-based targets: goal is < 1 micromolar IC50/EC50	**Surpasses criteria**; functional cellular assays: pTie2-ELISA, HUVEC-cells with IC_{50} 1.3 nM; NanoBRET™ assay with IC50 0.7 nM
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	**n/a**
Neg ctrl: *in vitro* potency - > 100 times less; Cell activity - >100 times less potent than the probe	**Surpasses criteria**; > 10,000 times less in biochemical assay (Tie-2); >1,000 times less in cellular assay (DDR1/2, STK10, EPHB6)

We ask for acceptance of Tie/DDR inhibitor BAY-826 as chemical probe, accompanied by BAY-309 as negative control which may allow to selectively study the biology of Tie/DDR signaling *in vitro* and *in vivo* as it does not target other angiogenic kinases

Tie/DDR probe BAY-826:

Acknowledgement

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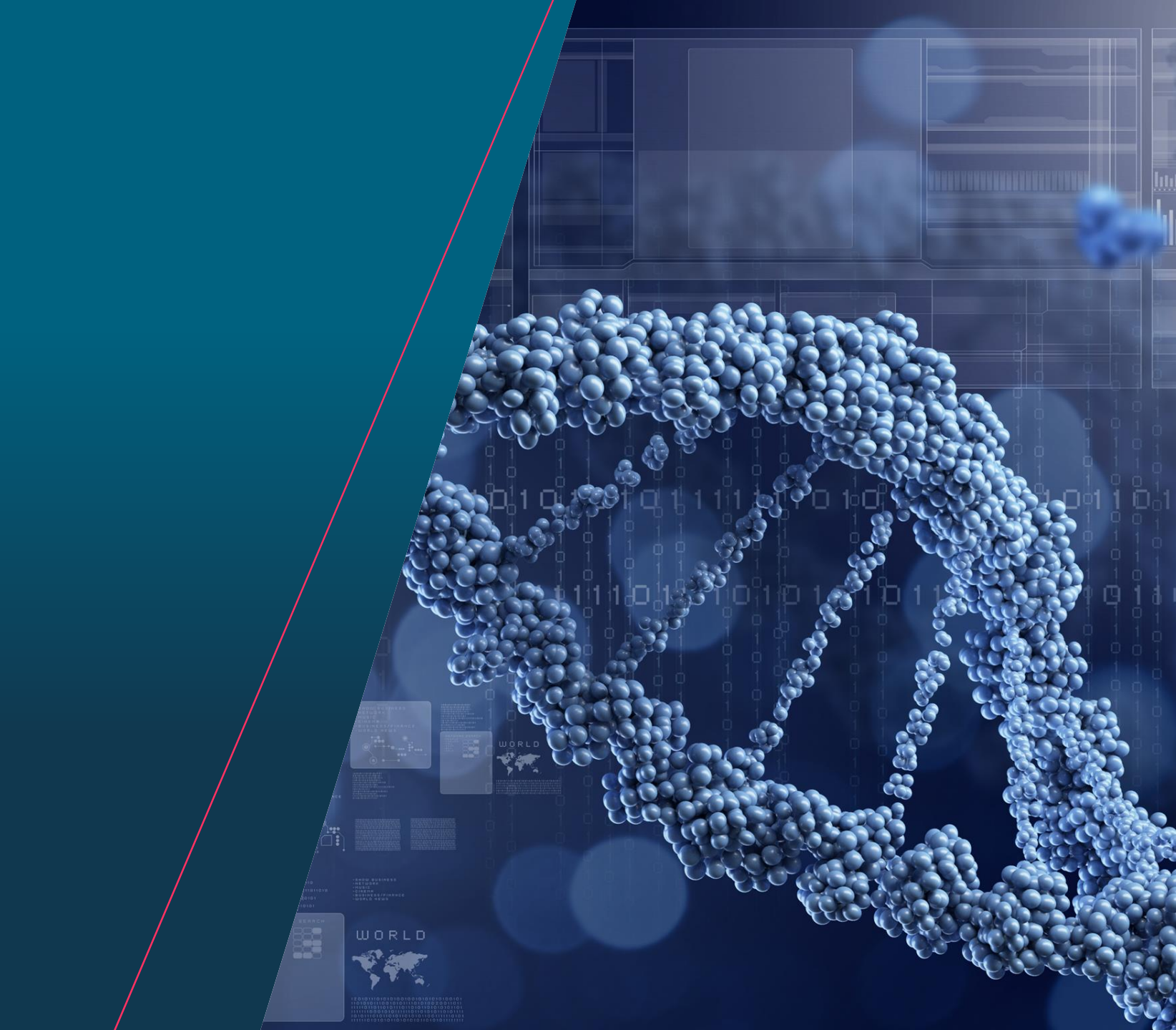
Oncotest
Julia Schüler
Kerstin Klingner

Thank You



Tie/DDR probe BAY-826 & negative control BAY-309:

NanoBRET™ assay data, SGC results