Science For A Better Life

Donated Probe BAY-876

GLUT1 inhibitor

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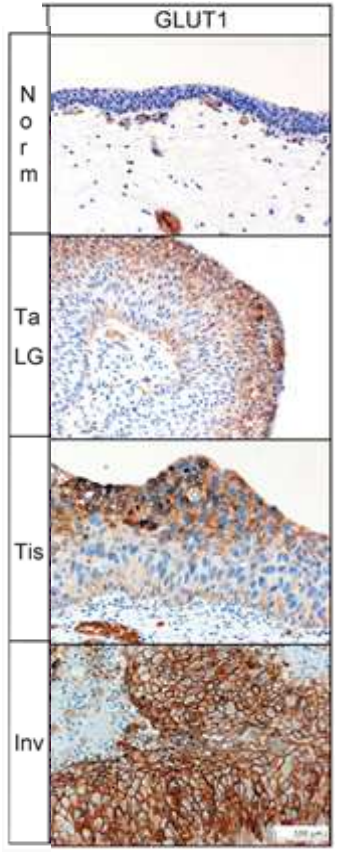
Introduction – Scientific Rationale

Key Points

- Most cancers exhibit increased glucose uptake and aerobic glycolysis, that is, the Warburg effect
- Glucose transporters are rate-limiting checkpoints, abnormally regulated in cancer
- Metabolic rewiring and increased glycolysis drives resistance to SOC therapy
- Glucose transporter 1 (GLUT1 / SLC2A1) is a high-affinity glucose transporter with a defined expression pattern in normal tissue
- GLUT-1 is one of the major glycolysis players up-regulated by hypoxia-inducible factor 1 alpha (HIF1α)

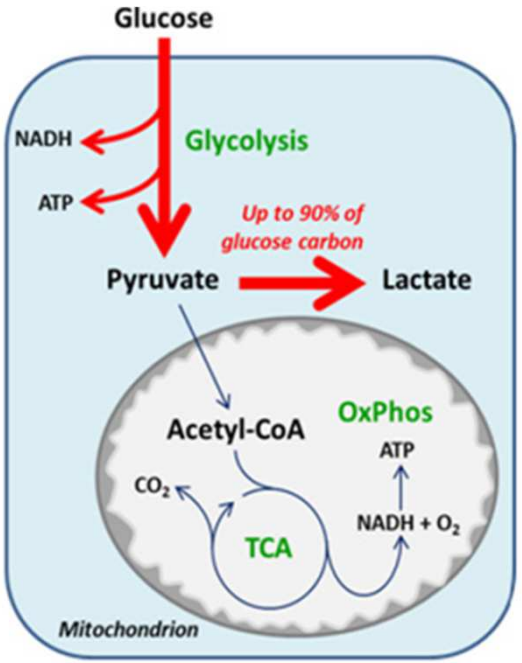


Carcinoma of the bladder, figure adapted from Reis et al. 2011

Introduction – Scientific Rationale

Rationale for a GLUT1 selective inhibitor

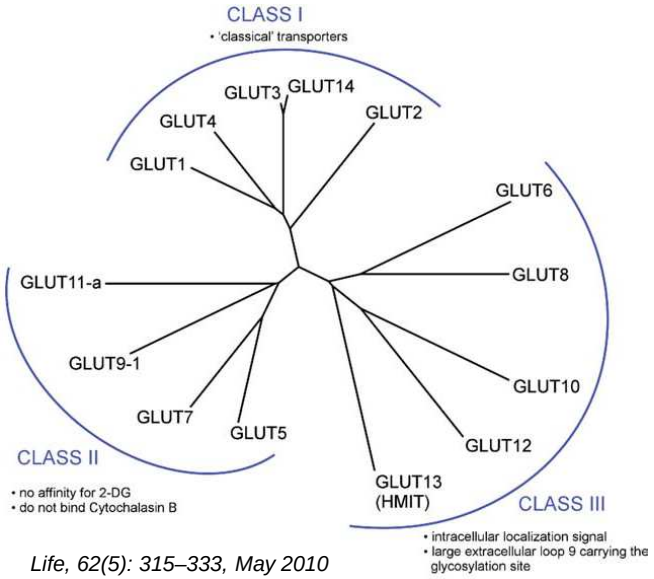
- Class I GLUT transporters have distinct physiological roles and share structural similarity
- GLUT-1 overexpression has been observed in multiple tumor indications
- GLUT-1 is one of the major glycolysis players up-regulated by hypoxia-inducible factor 1 alpha (HIF1α)
- Other Glucose transporters are additionally expressed in heart, muscle, liver, kidney, pancreas (beta cells)
- panGLUT inhibtitors have strong side effects in mice at efficacious dose (brain, heart)



Carcinoma of the bladder, figure adapted from Reis et al. 2011

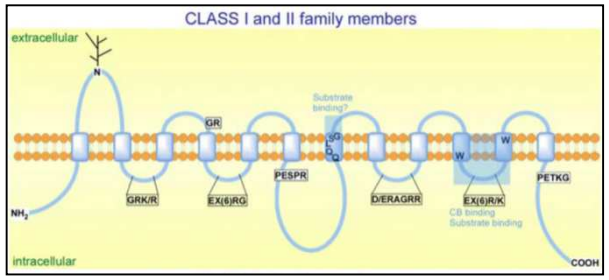
Specifically blocking GLUT1 should target tumors while minimizing the risk of side effects

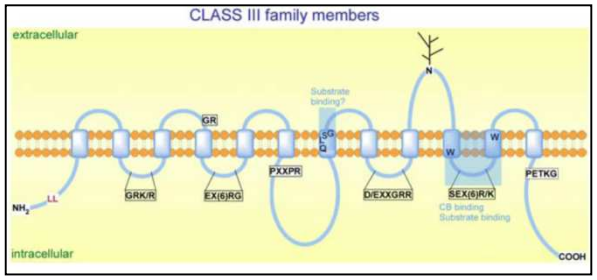
Introduction – Rationale



Life, 62(5): 315–333, May 2010

Protein Class I	Km (mM)	Major sites of expression	Proposed function	Homology Human/ Mouse
GLUT1	3-7	Ubiquitous, overexpressed in tumors	Transport across blood tissue barriers	96.3
GLUT2	17	Liver, kidney, small intestine, β-cells	„Glucose sensor" in pancreatic β-cells Transport across blood liver barrier	81.6
GLUT3	1.4	Neuronal cells	Neuronal transport	83.3
GLUT4	6.6	Muscle, fat, heart	Insulin-regulated transport	95.3

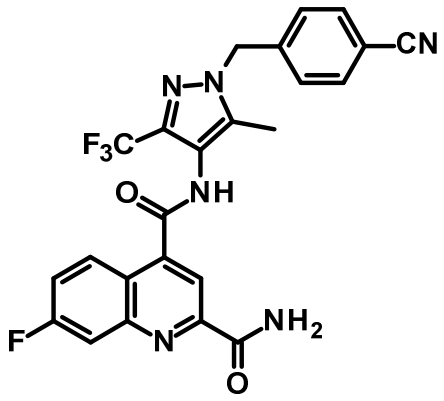




- **class I and II: N-linked glycosylation site at 1st exofacial loop between TM1 an TM2**
- **class III: shorter loop 1; glycosylation site at larger loop 9**

⇨ **Selectivity against GLUT2, GLUT3 and GLUT4 regarded necessary for therapeutic window**

Probe BAY-876 – Profile



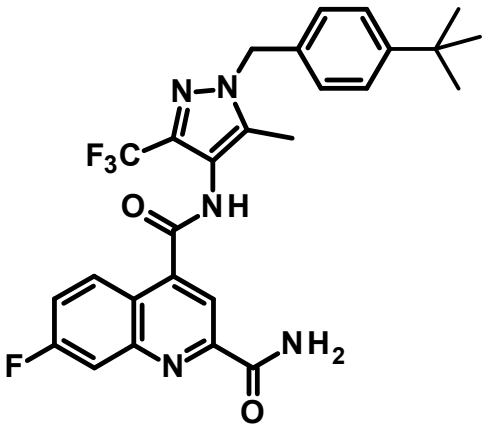
BAY-876

Lead-like properties	
$MW_{corrected}$	441.2 g/mol
TPSA	127 Å²
Measured logD (pH 7.5)	2.7
Calculated logD (pH 7.5)	2.6
Solubility (pH 6.5)	0.8 mg/L
Solubility (Tween80/Soya 5:1)	1610 mg/L
Stability in r/h plasma	stable

Highly potent, cellularly active and very selective		
IC_{50} GLUT-1		**2 nM**
Selectivity	**IC_{50} GLUT-2**	**9.4 µM**
	IC_{50} GLUT-3	**1.6 µM**
	IC_{50} GLUT-4	**270 nM**
IC_{50} Cellular mechanistic assay (Glucose uptake inhib. / HeLa-MaTu)		**3.2 nM**
Selectivity Kinases (# = 18)		> 20,000 nM
Selectivity Eurofins Panel (# = 68)		> 5,000 nM Adenosine A3 : IC_{50} = 1,140 nM Prostaglandin EP4 : IC_{50} = 1,120 nM

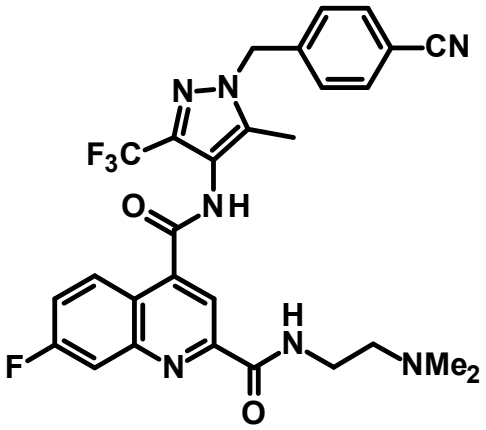
Promising in vitro / in vivo pharmacokinetic profile				
CL_{int} Hepatocytes	**Rat**	1.9 L/h/kg	**Rat PK in vivo**	
CL_{int} Microsomes	**Mouse**	0.93 L/h/kg	**Cl_b**	0.33 L/h/kg
	Human	0.18 L/h/kg	**V_{ss}**	0.79 L/kg
Caco2	**P_{app} AB**	78 nm/sec	**$t_{1/2}$**	4.9 h
	Efflux ratio	2.5 fold	**F**	85 %

Potential Negative-Probe
BAY-588 & BAY-480 – Basic Information



BAY-588

Basic information		
IC_{50} GLUT-1		**1.2 µM**
Selectivity	**IC_{50} GLUT-2**	**> 10µM**
	IC_{50} GLUT-3	**5.5 µM**
	IC_{50} GLUT-4	**0.5 µM**
$MW_{corrected}$		472.3 g/mol
TPSA		103 Å^2
Measured logD (pH 7.5)		3.9
Solubility (pH 6.5 / from DMSO sol.)		< 1 mg/L



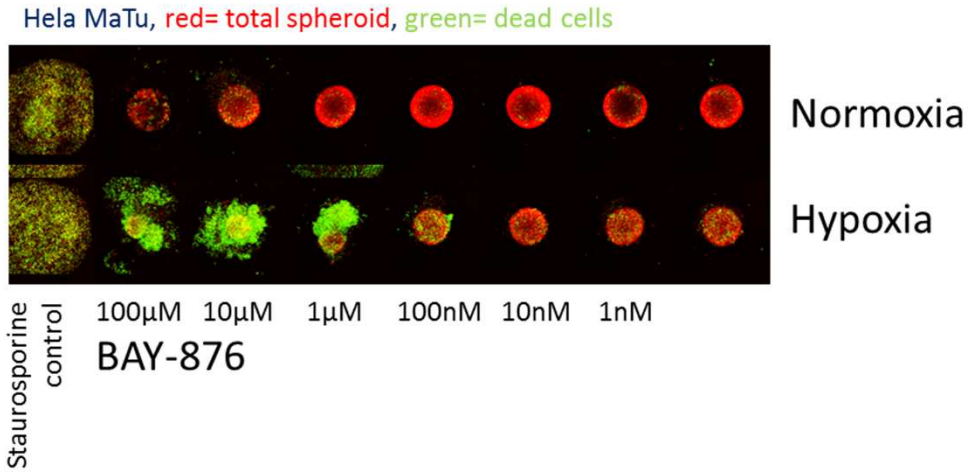
BAY-480

Basic information		
IC_{50} GLUT-1		**2.6 µM**
Selectivity	**IC_{50} GLUT-2**	**> 10µM**
	IC_{50} GLUT-3	**> 10 µM**
	IC_{50} GLUT-4	**2.8 µM**
$MW_{corrected}$		512.3 g/mol
TPSA		116 Å^2
Measured logD (pH 7.5)		2.4
Solubility (pH 6.5 / from DMSO sol.)		1.9 mg/L

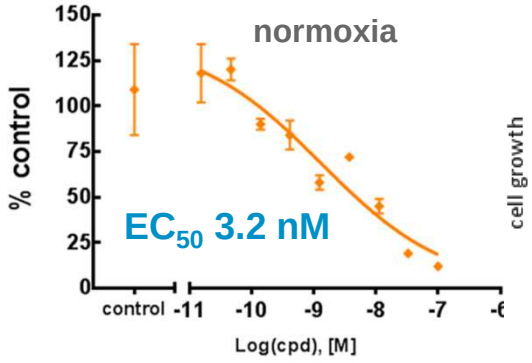
Initial Pharmacology of BAY-876 in HeLa-MaTu Cells

In vitro pharmacology of BAY-876

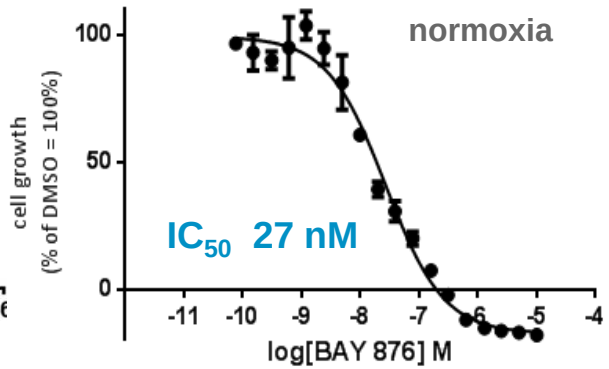
Induction of cell death under hypoxia in 3D assay



Inhibition of glucose uptake

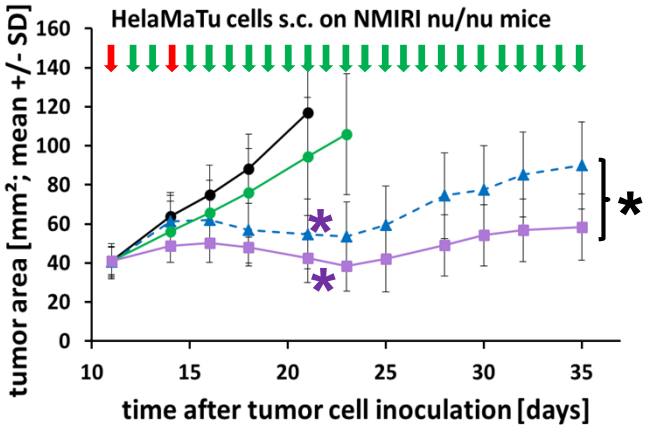


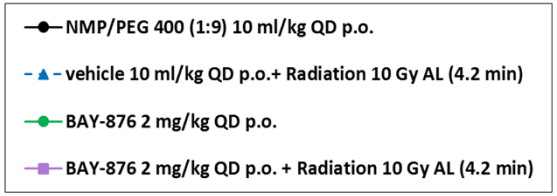
Inhibition of proliferation (2D)



BAY-876 *in vivo*

Additive anti-tumor efficacy of BAY-876 in combination with radiation *in vivo*





* P = 0.016 (t-test)
* P < 0.05 vs. vehicle (Dunnett´s method)
Radiation
BAY-876 treatment

Summary / Conclusion

- BAY-876 is a selective GLUT1 inhibitor meeting all chemical probe criteria:
  - Biochemical activity IC_{50} at 2 nM
  - On-target cellular activity (glucose uptake assay in HeLa-MaTu cell line) at 3.2 nM
  - Selectivity against family members was tested
- Selectivity in kinase and receptor panel was performed
- Additionally, two structure related compounds with low activity were identified and will be provided
- ***BAY-876 is a highly potent, cellular active and very selective inhibitor with a promising in vitro / in vivo pharmacokinetic profile***
- ***We would therefore provide the GLUT1 inhibitor BAY-876 and an inactive/negative control as a chemical probe***

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