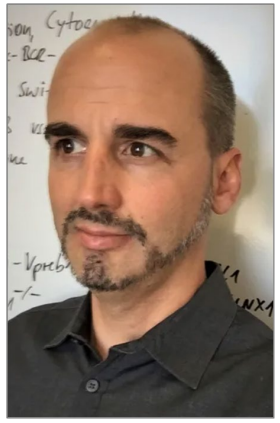
Cutting-edge therapy for refractory B-cell malignancies and autoimmune diseases

Developing a New Generation of Orally Bioavailable Selective GSK3B Inhibitors

World Class Team in B-Cell Malignancy and Drug Development



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Yale SCHOOL OF MEDICINE

Yale CANCER CENTER

A Comprehensive Cancer Center Designated by the National Cancer Institute

Urgent need for enhanced therapies in Refractory B-cell malignancies

- **Refractory B-Cell Malignancy** is a form of B-Cell lymphoma or leukemia that **does not respond to standard treatments or relapse quickly after initial response**. This cancer continues to progress despite therapeutic efforts, **making it challenging to manage and control**.

~300,000

People are estimated to have a B-Cell Malignancy in the USA

20-75%

Of patients will be refractory to or relapse after initial treatment

$200-300K

The mean costs per patient for adverse effects from second, third, or fourth-line treatments (each).

The current FDA-approved therapies for second, third, and fourth-line treatments **do not have favorable safety profiles** and there is **room for improvement in terms of efficacy**. All Cancer **treatments require stay at healthcare facility/assistance** due to continuous infusion drug delivery

The journey of a patient with refractory B-cell lymphoma

First in Line Therapy

Example:

Chemotherapy

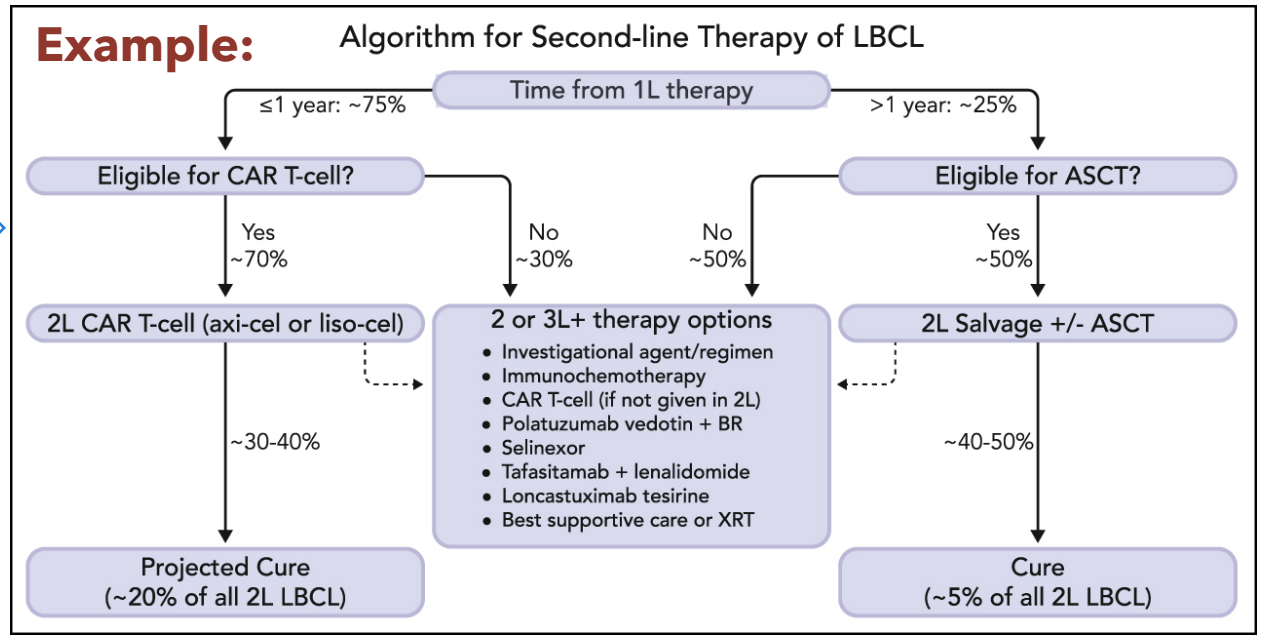
CHOP:
Cyclophosphamide
Doxorubicin
Vincristine
Prednisone

+ Rituximab (Monoclonal Antibody)

20-75% Not Cured

Therapy Options for Refractory B-Cell Malignancies

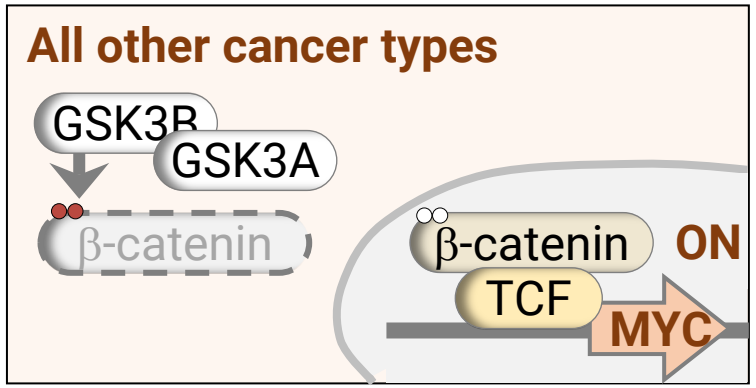
Example:



***There is a need for more effective and safer 2nd or 3rd Line Therapy Options for Refractory B Cell Lymphoma**
(Highly Toxic and Severe Long-Term Effects, Need to Be at The Hospital)

Our Discovery:

GSK3B-mediated degradation of β-catenin -a unique vulnerability of B-cells



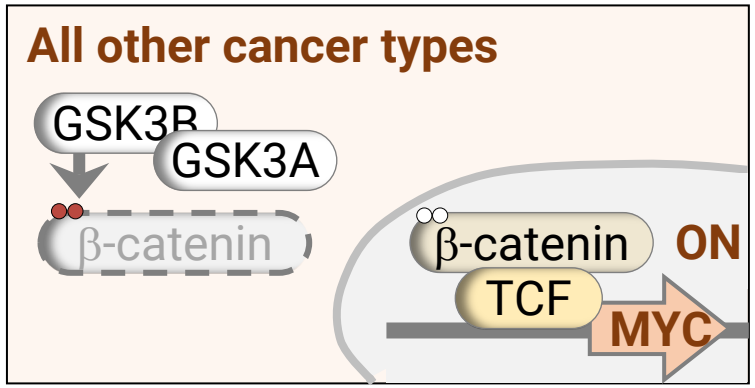
GSK3A/B are the kinases that induces degradation of β-catenin

β-catenin pairs with **TCF** for activation of MYC:

Cells proliferate

Our Discovery:

GSK3B-mediated degradation of β-catenin -a unique vulnerability of B-cells



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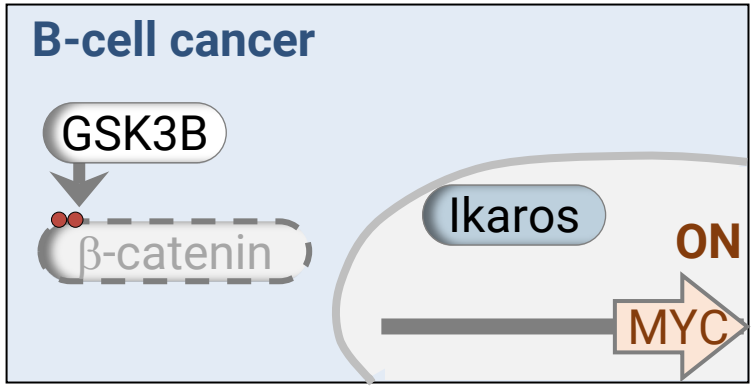
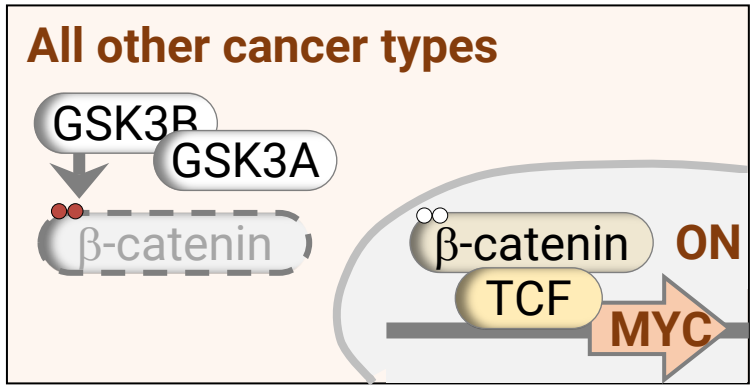


In B-cells, β-catenin is barely detectable, efficiently degraded by GSK3B.

Our Discovery:

GSK3B-mediated degradation of β-catenin -a unique vulnerability of B-cells



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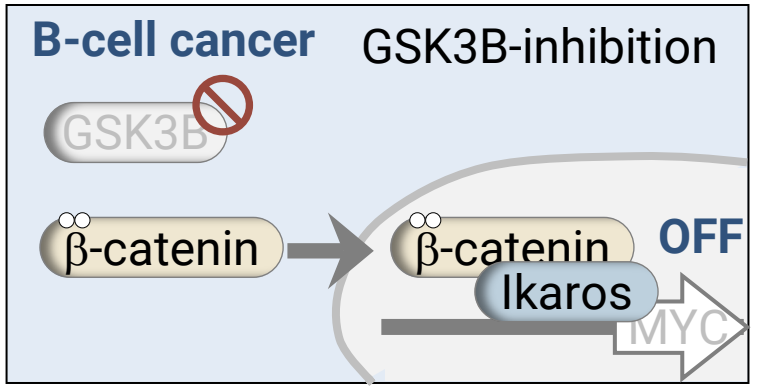


β-catenin pairs with **Ikaros** for repression of MYC:

Cells stop dividing and die

1 R01 CA282877-01

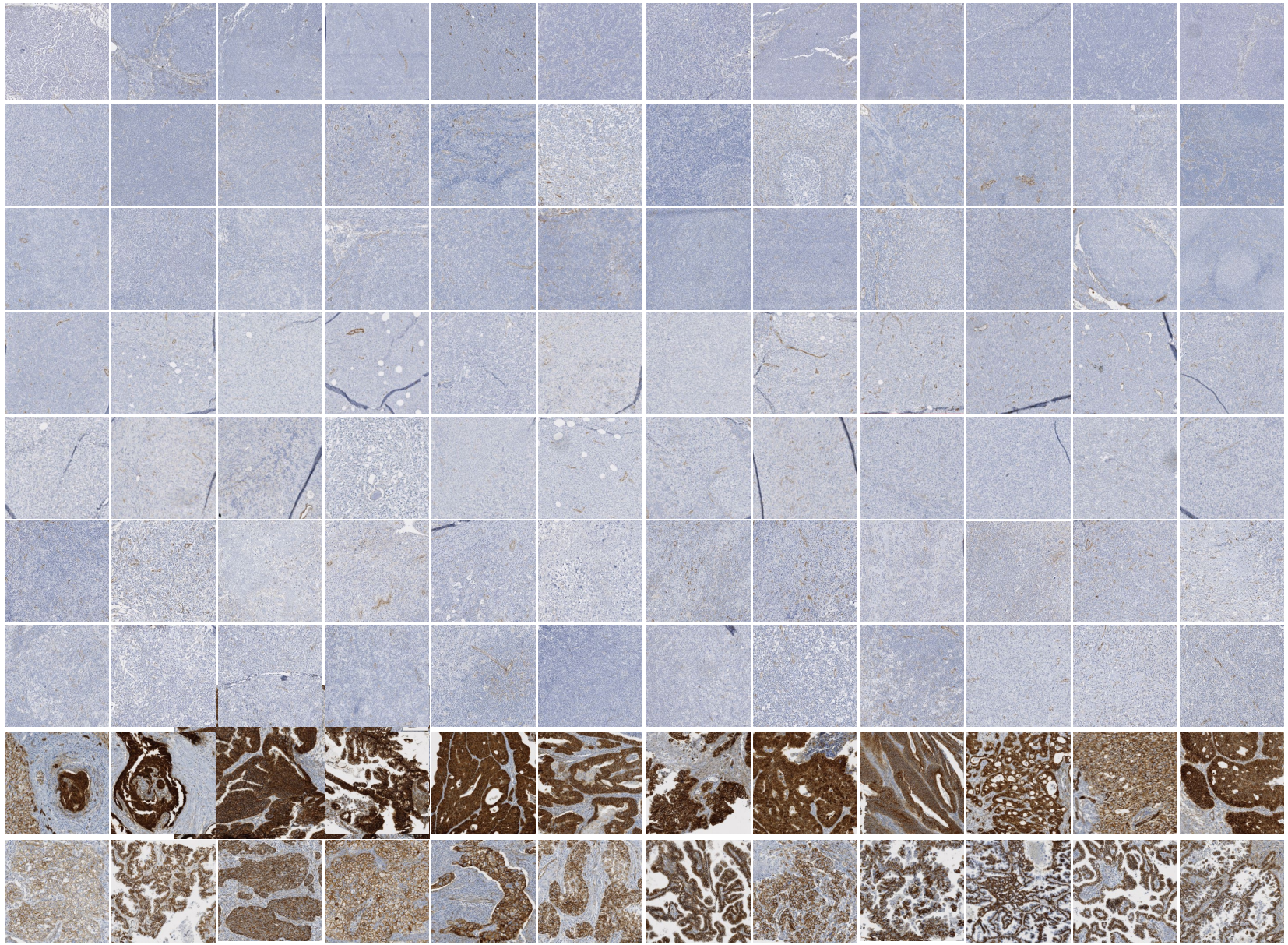
Project Title
Targeting GSK3B in refractory B-cell malignancies

PI Name
Müschen, Markus

Application

Award Document Number: RCA282877A
FSR Accepted Code: N
Snap Indicator Code:
Impact Score: 10
Percentile: 1.0

B-cell malignancies are uniquely efficient at β-catenin degradation



Mantle cell Lymphoma

Follicular Lymphoma

DLBCL

Hodgkin's Lymphoma

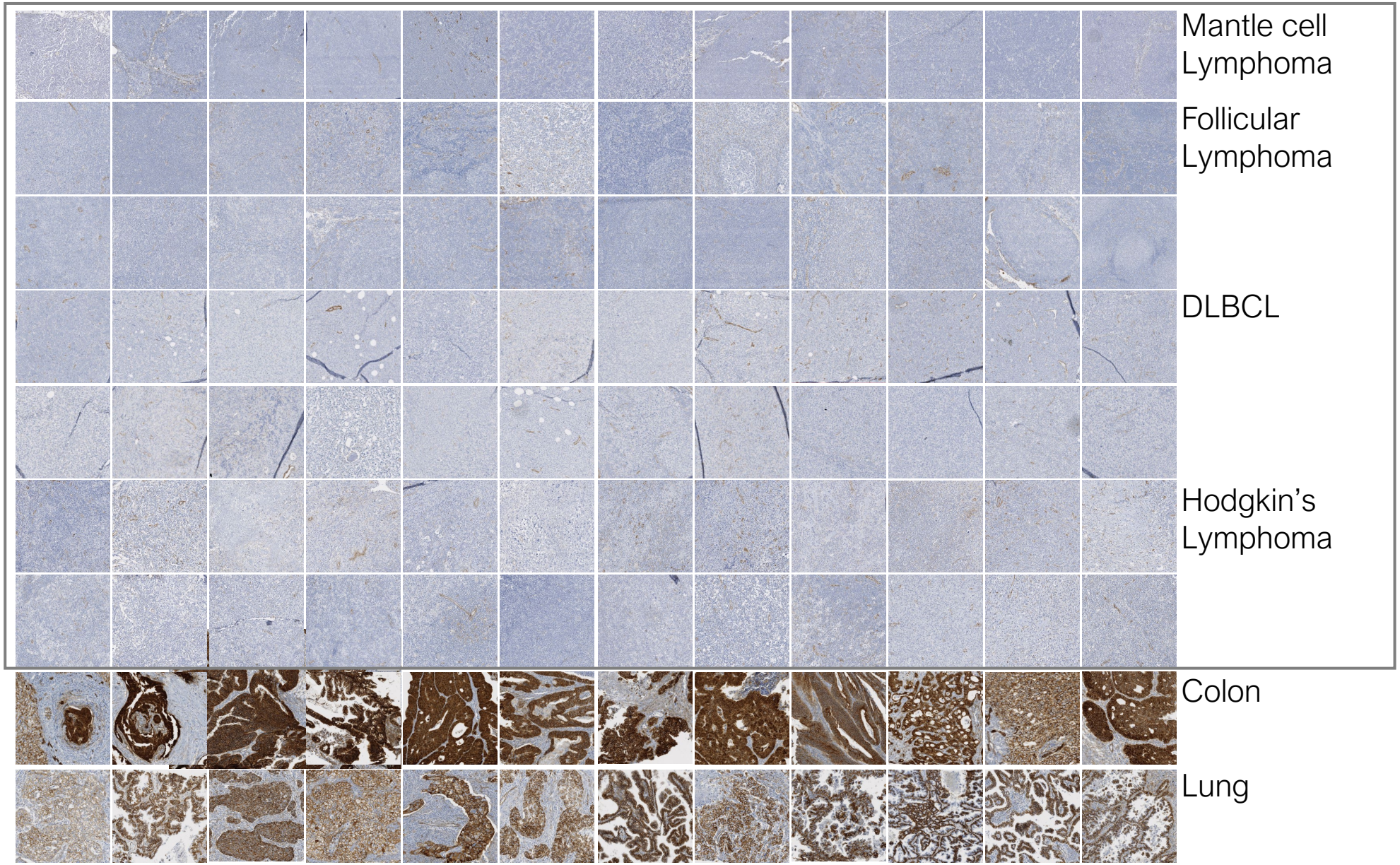
Colon

Lung

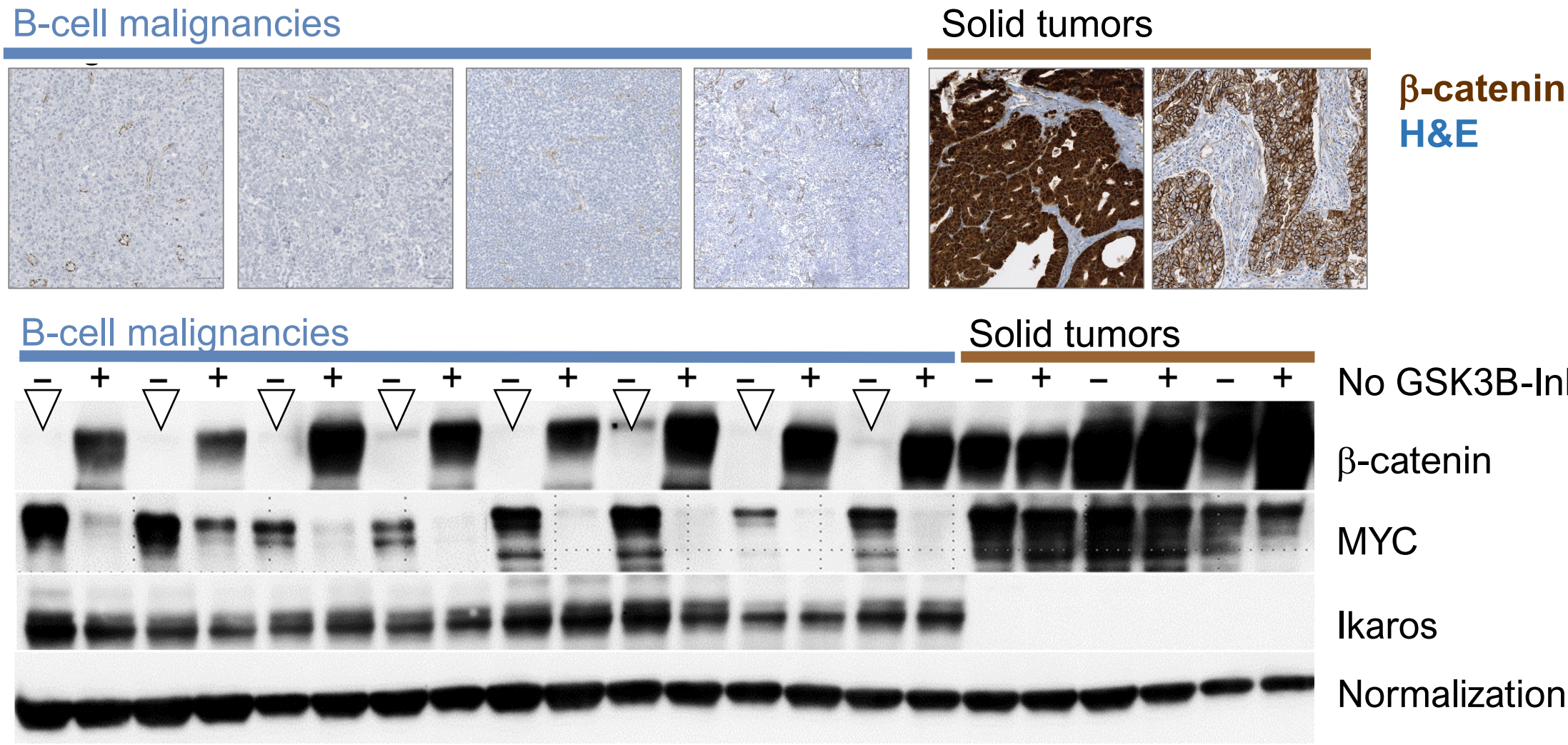
H&E

β-catenin

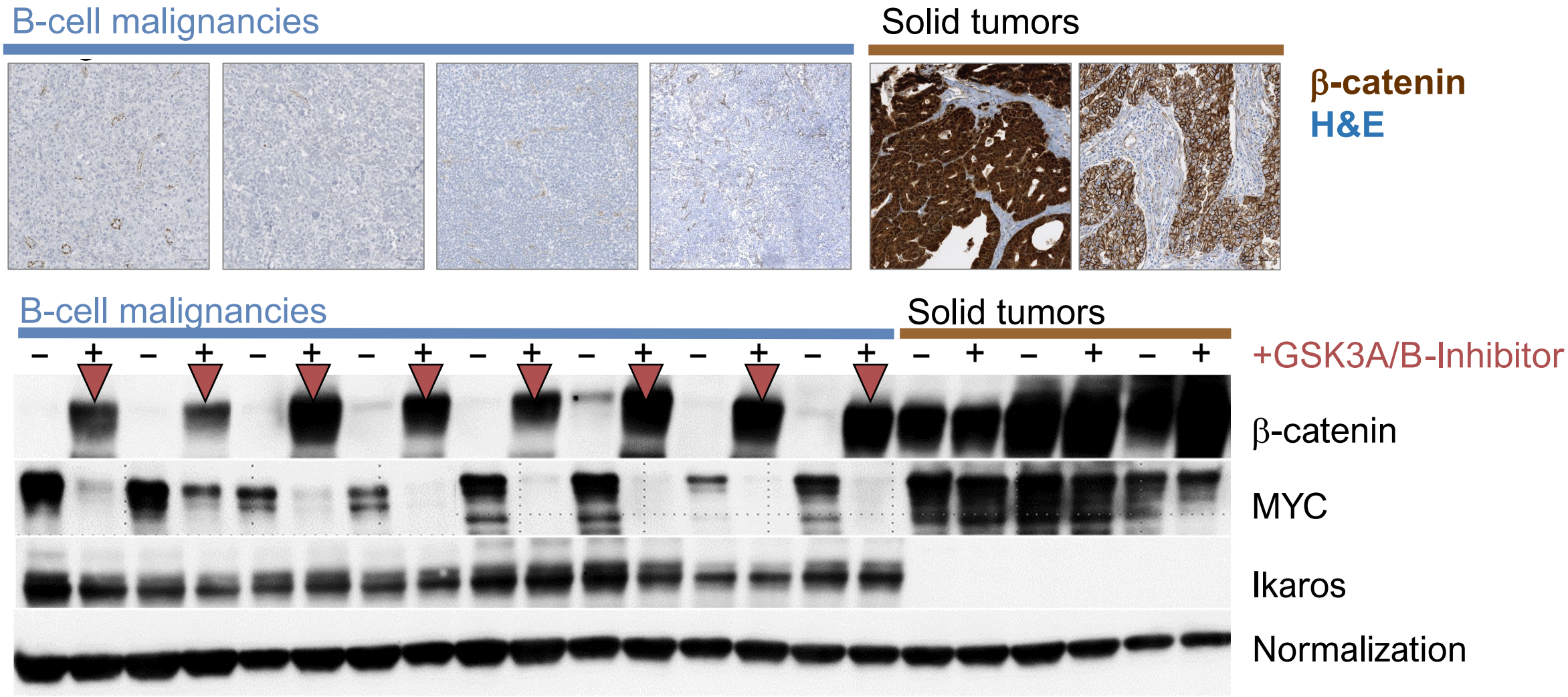
B-cell malignancies are uniquely efficient at β-catenin degradation



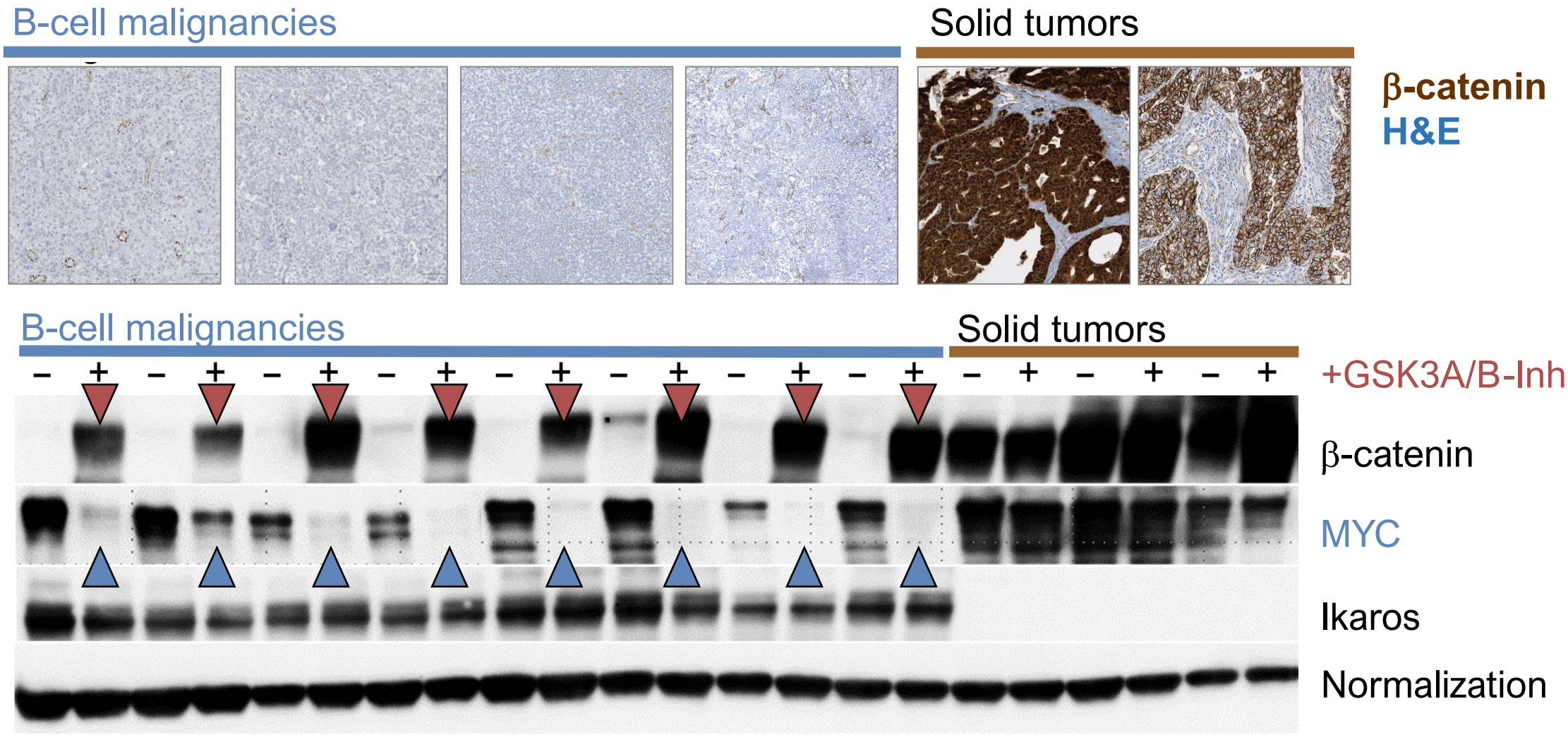
In the absence of GSK3B-inhibitor: β-catenin protein efficiently degraded



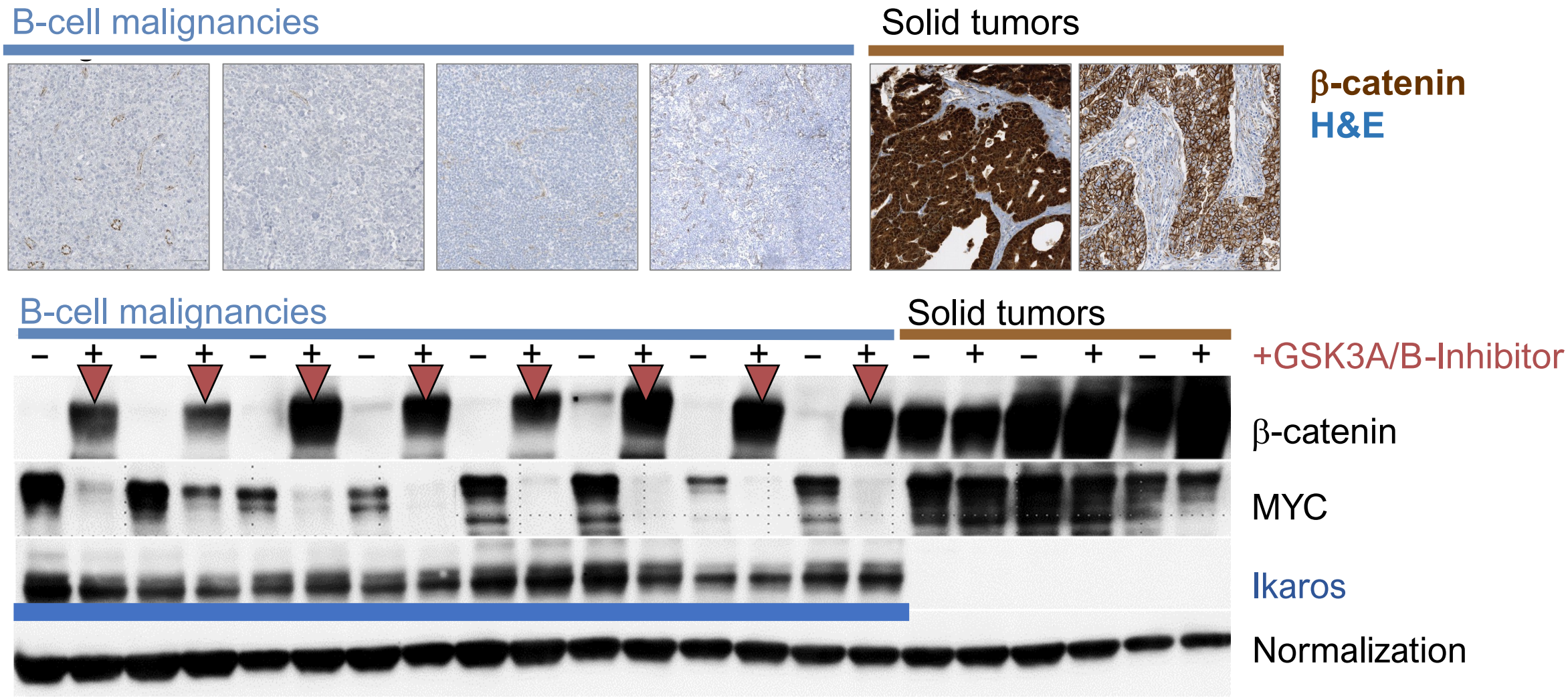
GSK3B-inhibitor disrupts β-catenin protein degradation: accumulation in B-cells



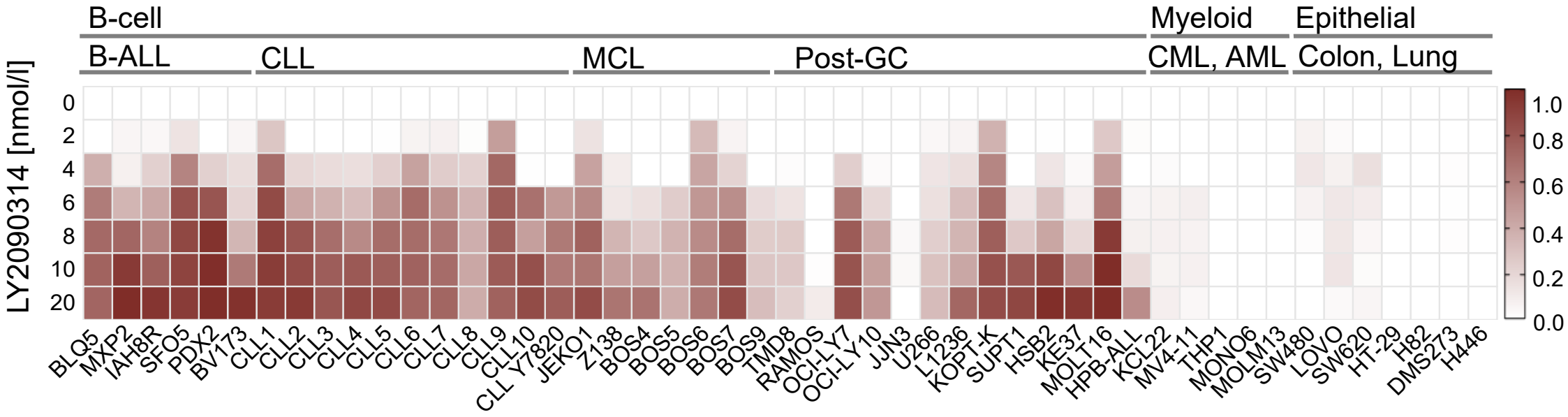
GSK3B-inhibitor disrupts β-catenin protein degradation and suppresses MYC

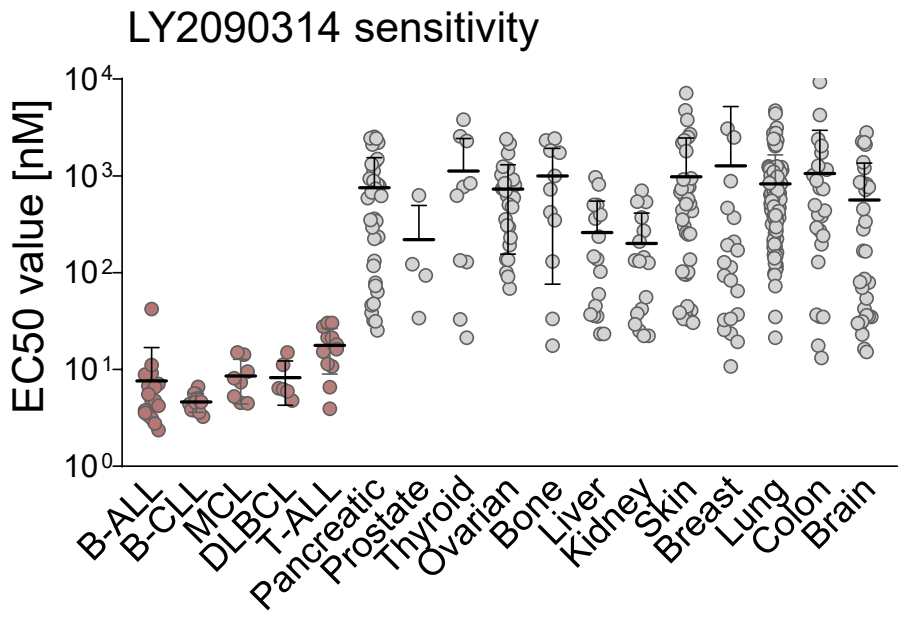


Only B-cells express Ikaros –required for MOA



GSK3B inhibitors for refractory B-cell malignancies





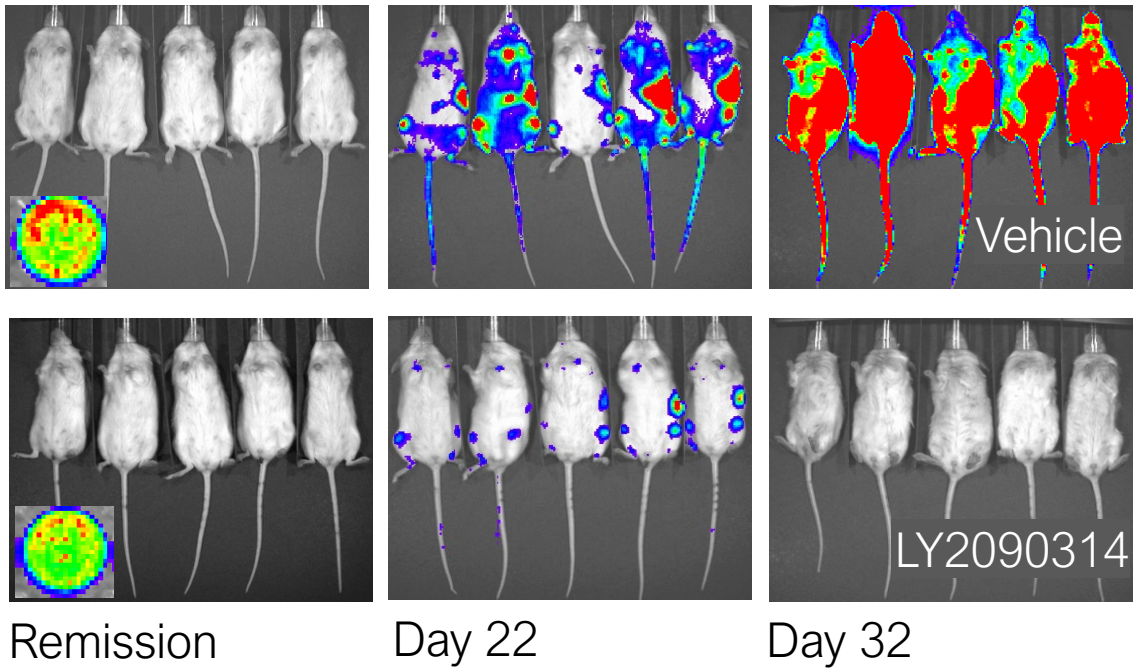
- GSK3B inhibitors are effective at low nanomolar concentrations in B-cell malignancies
- EC50 values in B-cell malignancies are ~400-fold lower than in other cancer types
- B-cell lymphomas with MYC-translocation are resistant to GSK3B inhibition

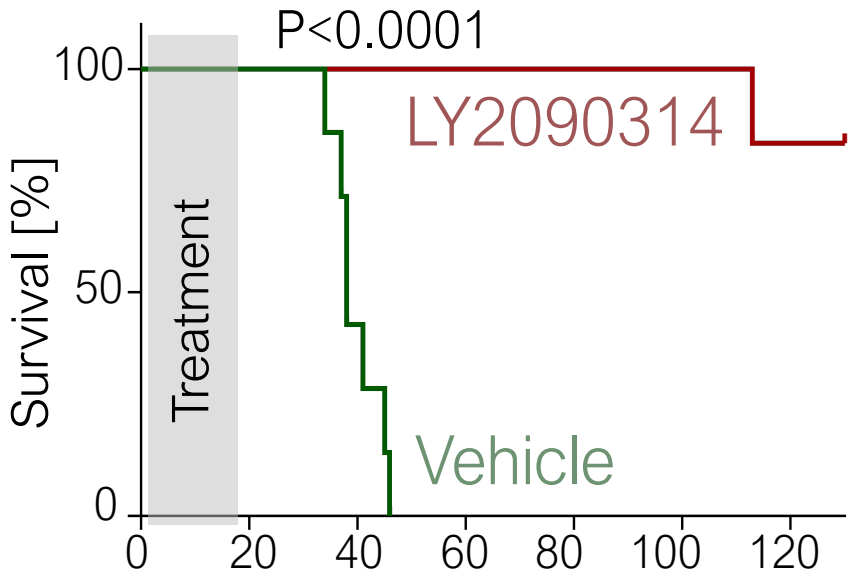
Our proposal: use GSK3B inhibitors in diseases caused by pathological B-cells

- Primary indication: refractory B-cell malignancies
- Secondary indication: B-cells and plasma cells in refractory autoimmune diseases

Our *in vivo* POC studies in PDX model support efficacy of GSK3B-inhibition

B-cell leukemia PDX





GSK3A/B dual kinase inhibitors in development demonstrate favorable safety & PK/PD profiles

Compound	Target	Indication	NCT Identifier	Outcome	Adverse effects
LY2090314	GSK3α GSK3β	Gastrointestinal cancer, pancreatic carcinoma	NCT01287520, NCT01214603, NCT01632306	Phase 1 and 2 No clinical responses Favorable PK/PD, safety profile	B-cell defect diarrhea
9-ING-41 Elraglusib	GSK3α	Advanced sarcomas, salivary gland carcinoma, pancreatic carcinoma, melanoma	NCT03678883, NCT05239182, NCT04239092, NCT05077800, NCT04906876, NCT03678883, NCT05116800, NCT04218071, NCT04832438, NCT05010629	Phase 1 and 2, no clinical responses, IND withdrawn, favorable safety profile	Hypoglycemia diarrhea
Tideglusib	GSK3α	Alzheimer's disease, myotonic dystrophy, supranuclear palsy, tooth repair (dentin)	NCT01350362, NCT00948259	Phase 1 and 2 No clinical responses, favorable PK/PD, safety profile	Hypoglycemia diarrhea
AZD1080	GSK3α GSK3β	Alzheimer's disease, Parkinson	Trial in Sweden, PMID: 23410232	Phase 1, favorable PK/PD, safety profile	B-cell defect diarrhea
CHIR99021 Laduviglusib	GSK3α GSK3β	NK-cell infusion for ovarian cancer, solid tumors, hearing loss	NCT03081780, NCT03213964, NCT03319459, NCT03616223	Phase 1 and 2 No clinical responses, favorable PK/PD, safety profile	B-cell defect diarrhea

…but require i.v. infusion and do not discriminate between GSK3A and GSK3B

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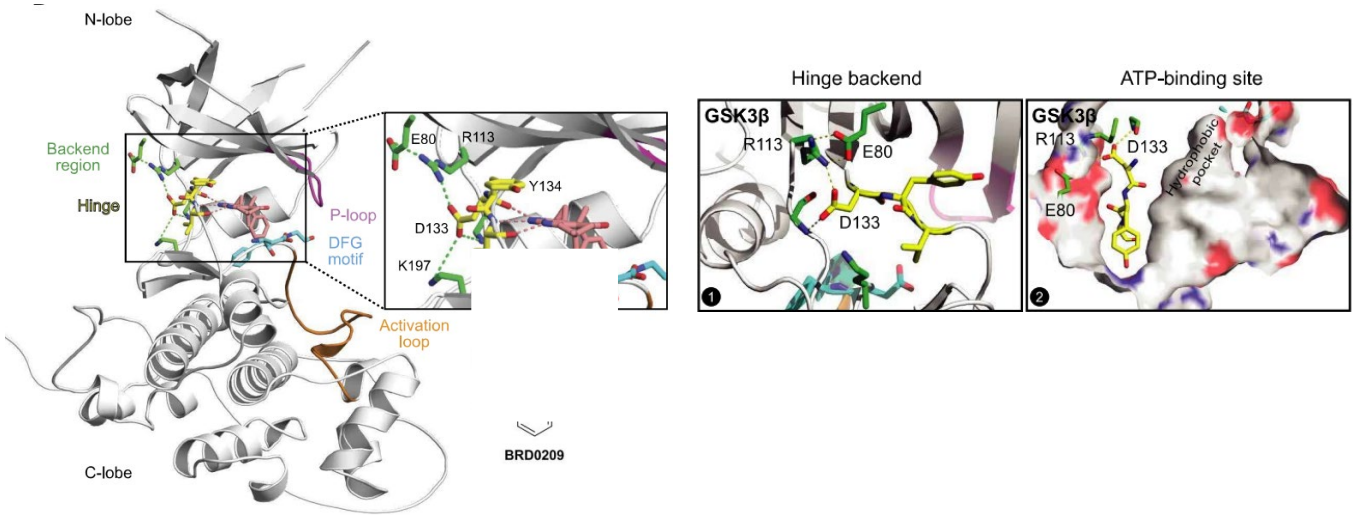
Key to developing paralog-specific GSK3B inhibitors to target the Asp^{133} ->Glu^{196} switch

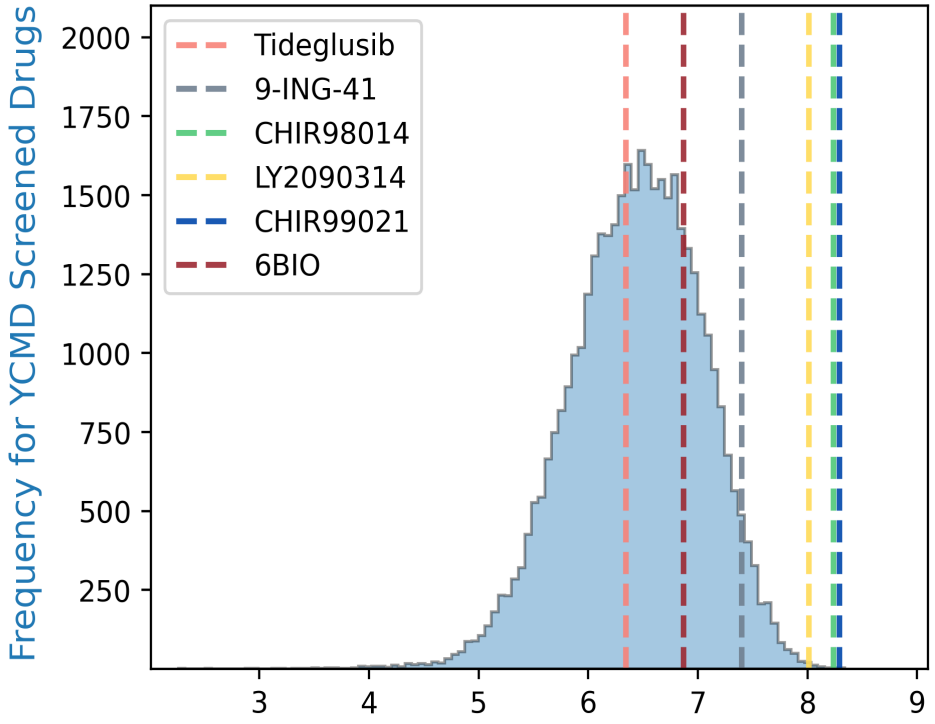
Novel Strategy:
Targeting "Switch" Instead of ATP-Binding Pocket



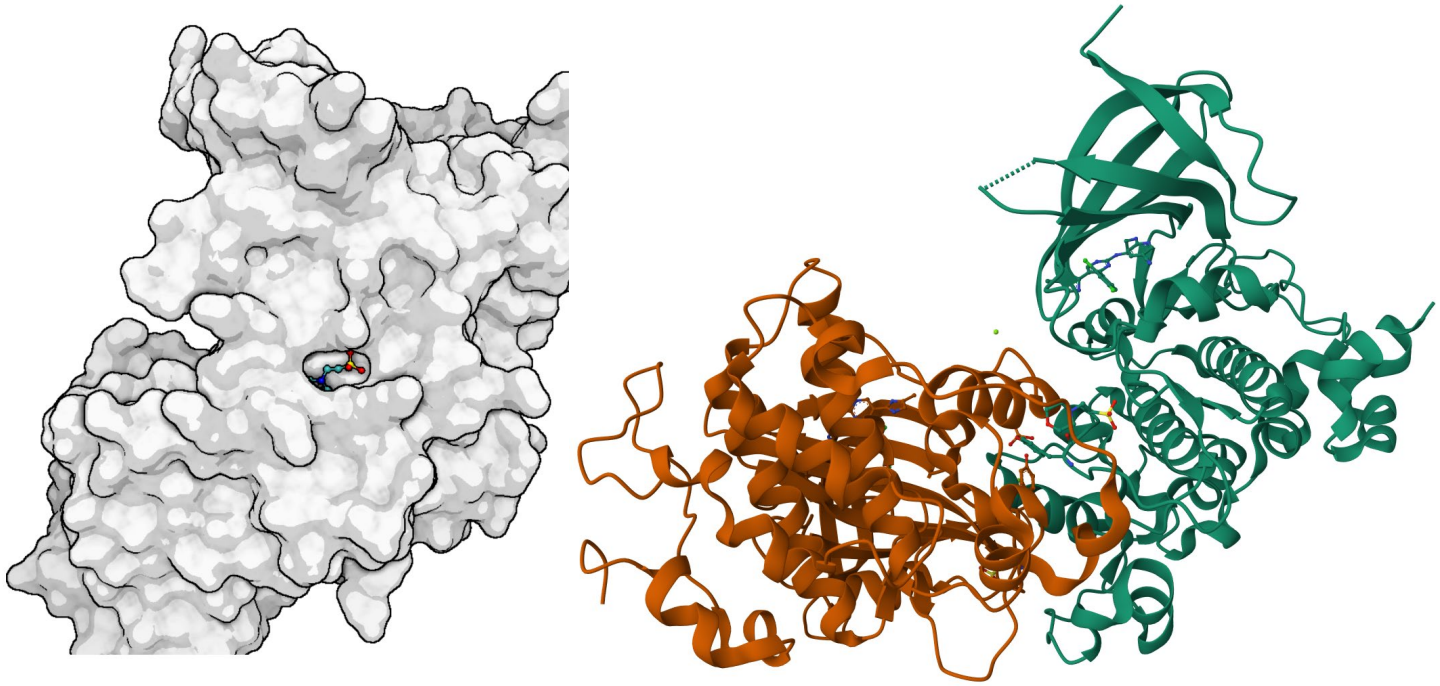
- The ATP Pocket (Pocket 1) homology is too similar between GSK3A and B, **often leading off-target effects**
- GSK3B implicated in other physiological pathways therefore inhibitor needs to be **reversible** to limit adverse effects
- **Targeting the "switch"** in the kinase hinge presents a rational design strategy to allow for specific GSK3B inhibition.
  - GSK3A: Glu^{196}
  - **GSK3B: Asp^{133}**

Our starting point for *in silico* screen:

- GSK3B cocrystal structures
- Structural basis for GSK3B vs GSK3A selectivity is known



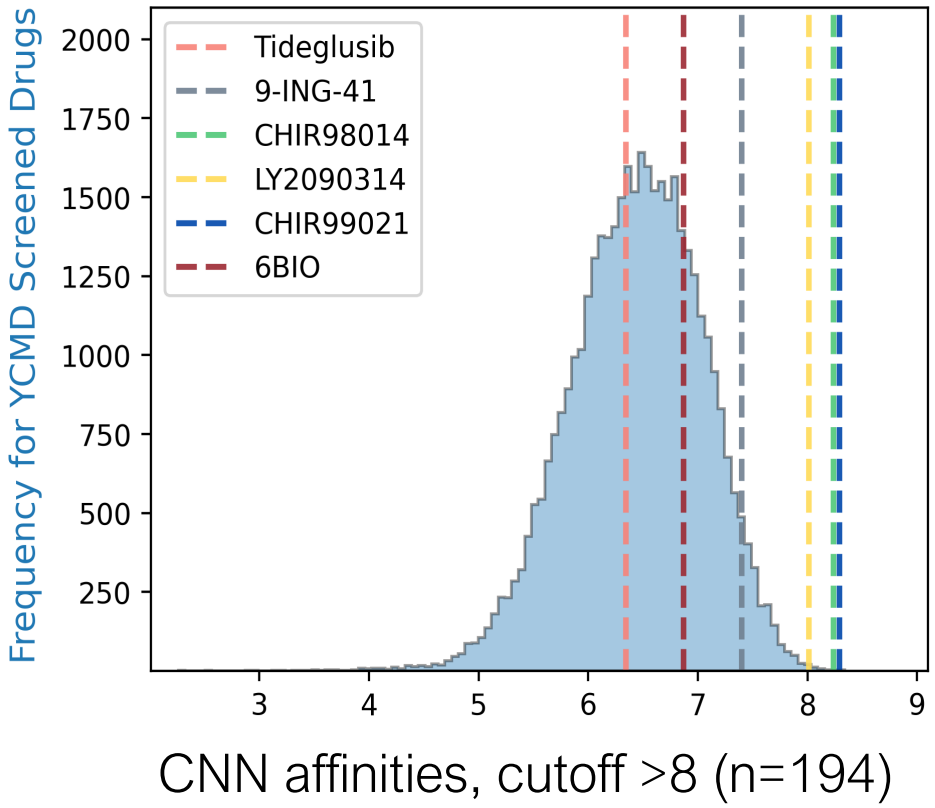
CNN affinities, cutoff >8 (n=194)



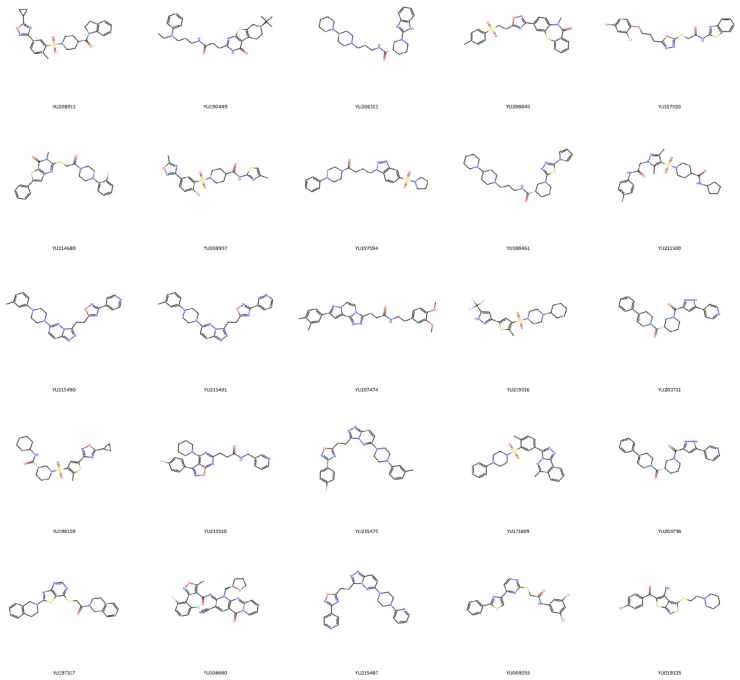
Co-crystal structure between GSK3B and CHIR99021
PDB 5HLN

HTS assay for optimized Class of GSK3B-selective, orally bioavailable Inhibitors

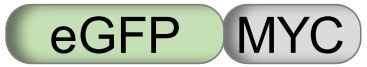
Positive control with existing GSK3 inhibitors



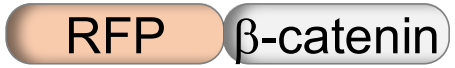
Design-away from non-selective tricyclic derivatives of a pyrazolo-tetrahydroquinoline scaffold (n=194, library of 460,000 compounds)



Proprietary GSK3B-inhibitor HTS assay:
Select for Accumulation of β-catenin and Repression of MYC

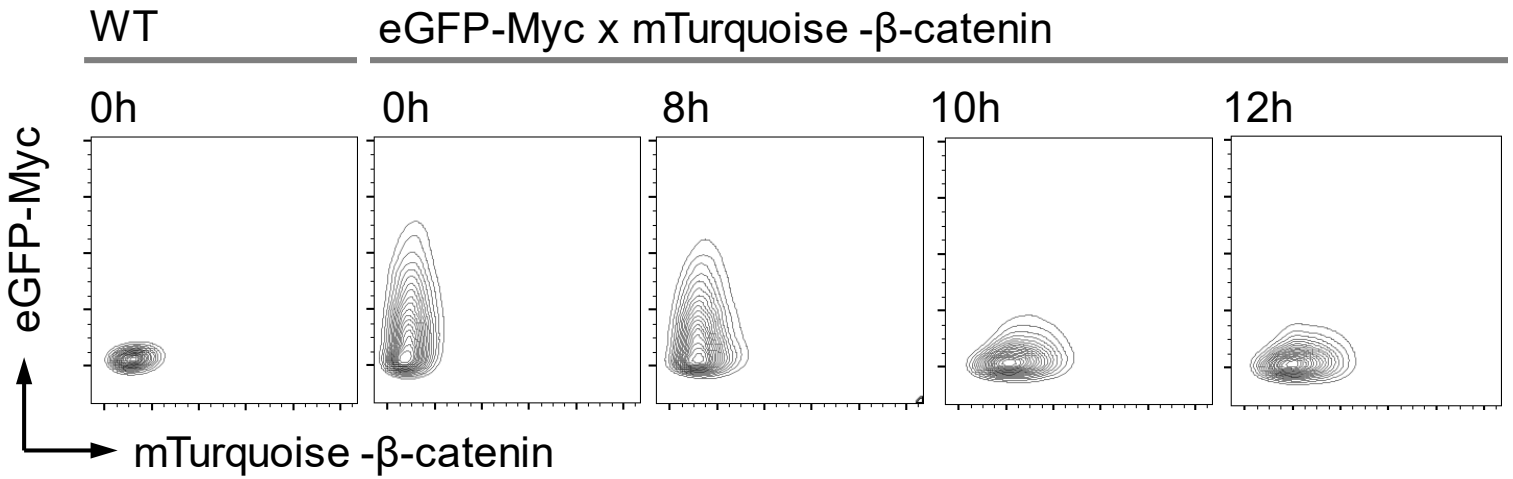


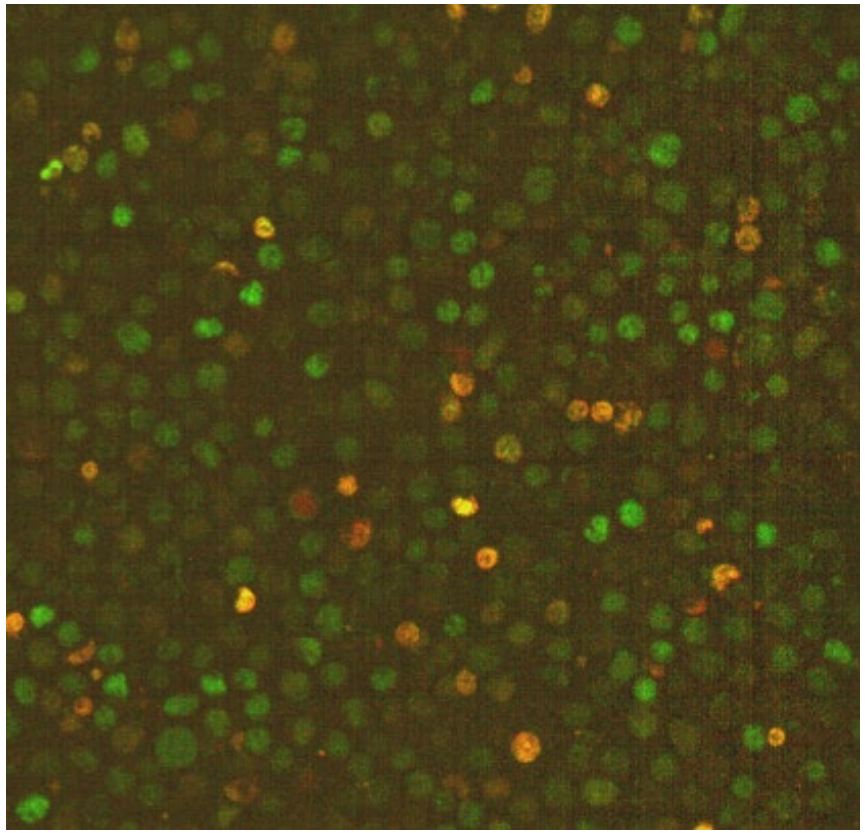
eGFP-MYC fusion

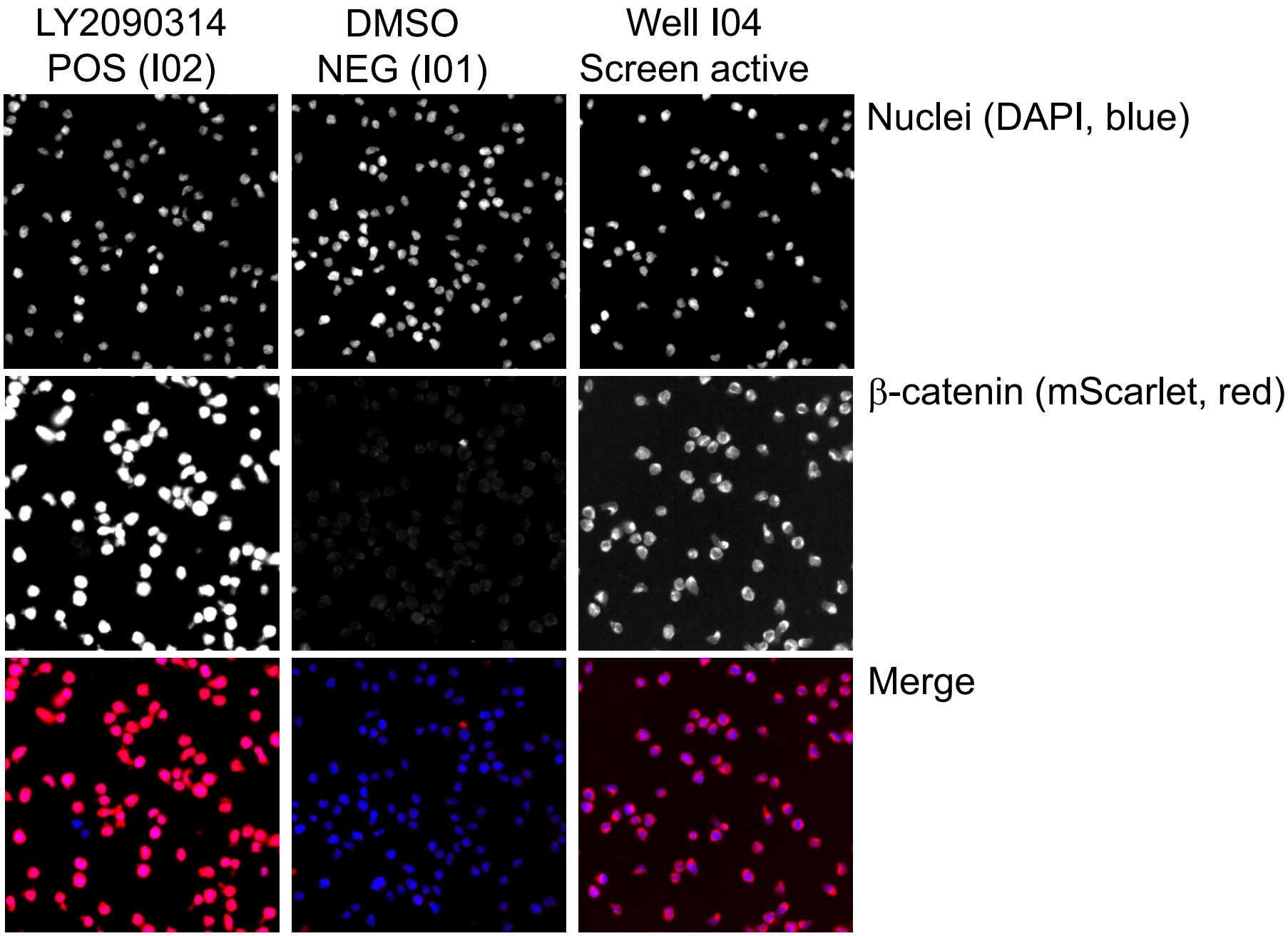


RFP-β-catenin

Validation with 10 nM LY2090314






LY2090314
POS (I02)
DMSO
NEG (I01)
Well I04
Screen active
Nuclei (DAPI, blue)
β-catenin (mScarlet, red)
Merge

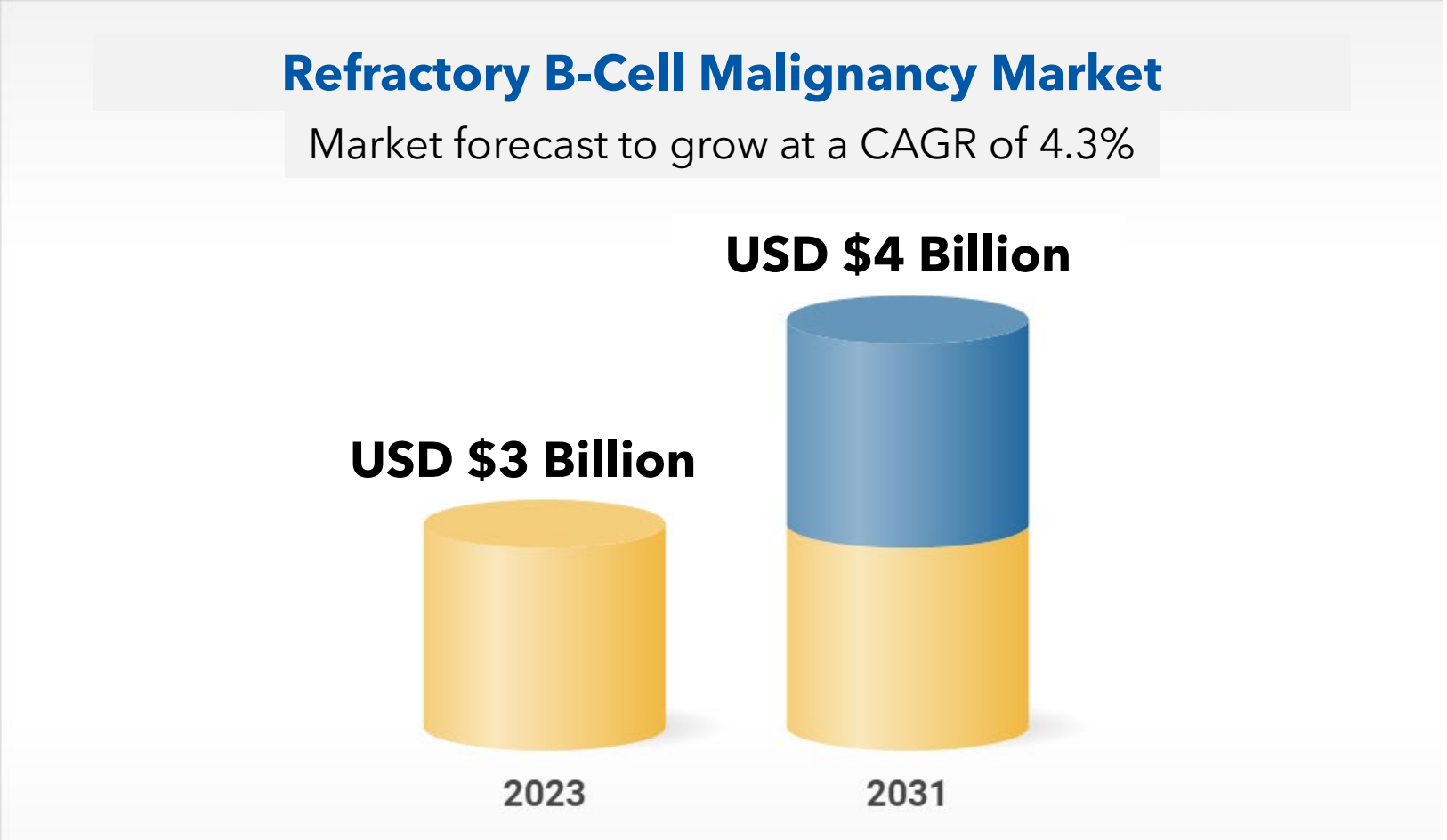
Currently approved therapies in **B-cell leukemia** show high response rates –but no oral delivery options

Company	Mechanism of Action	Delivery	Complete Response Rate	Objective Response Rate
Pfizer BESPONSA inotuzumab ozogamicin	Anti-CD22 ADC	IV	70%	78%
NOVARTIS KYMRIAH (tisagenlecleucel) Suspension for IV infusion	Anti-CD19 CAR T-Cell	IV	68%	86%
GILEAD TECARTUS (brexucabtagene autoleucel) Suspension for IV infusion	Autologous Cell Therapy	IV	57%	87%

Novel Therapies are Still Being Developed in the **B-Cell Lymphoma** Space –but no oral delivery options

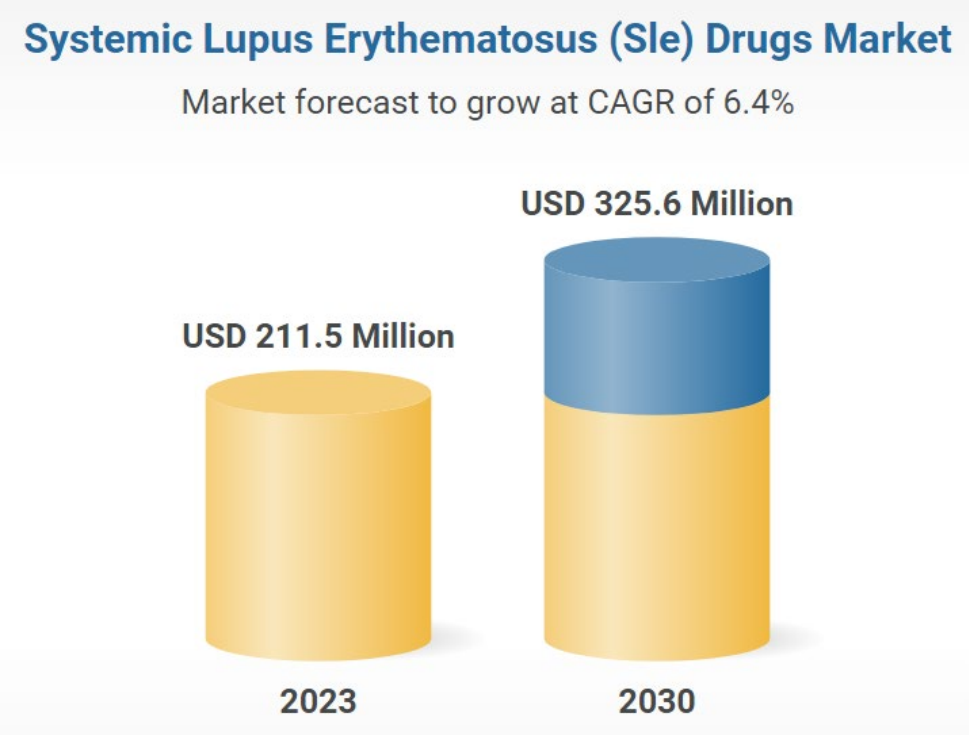
Company	Mechanism of Action	Stage	Indication
Bristol Myers Squibb®	Interferon Pathway Modifier	Phase II	R/R B Cell Malignancies
Bristol Myers Squibb®	Anti-CD19 CAR T-Cell	Phase I	B-Cell Malignancies
Roche	CD20xCD3 bispecific AB	Phase II	R/R Non-Hodgkin's Lymphoma
PRECISION BIOSCIENCES	Allogenic CAR T-Cell	Phase I/II	R/R Non-Hodgkin's Lymphoma, R/R ALL

Refractory B –cell malignancy market size is growing



Secondary indication:

B-cell autoimmune diseases landscape, market size is growing



Drug	Company	Target	Phase
Anifrolumab	AstraZeneca	IFN/Interferon pathway	Approved
Obinutuzumab	Roche	Anti-CD20 mAb	III
Dapirolizumab pegol	Biogen	Anti-CD40L (PEG-conjugated)	III
BIIB059	Biogen	Anti-BDCA-2 mAb	III
Telitacicept	RemeGen	Fusion BLyS/APRIL inhibitor	II/III
Daxdilimab/HZN-7734	HORIZON / VIELABIO	Ant-ILT7 mAb	II
Fenebrutinib	Genentech	BTK inhibitor	II
PF-06700841	Pfizer	JAK1 and TYK2 inhibitor	II
AMG 570	AMGEN	bsAb (ICOSLxBAFF)	II
NKTR-358	Lilly / NEKTAR	IL-2	II
Nipocalimab	Johnson & Johnson	Anti-FCRn mAb	II
BMS-986256	Bristol Myers Squibb	TLR 7/8 antagonist	II
KZR-616	KEZAR LIFE SCIENCES	Immunoproteasome Inhibitor	I/II
Lanalumab	NOVARTIS	Anti-BAFF	II

Developing a new class of orally bioavailable and selective GSK3B Inhibitors

MILESTONE 1

Development of Proprietary Compound Screening Assay

Non-provisional patent filed

COMPLETED

MILESTONE 2

- Primary screen to identify 10 compounds from 194 in-silico selected candidates
- Secondary screen utilizing CADD, SAR, fragment-based screens

IN-PROGRESS

MILESTONE 3

- Screen for GSK3B specific hits

Filing of Provisional Patents for new compounds

FUTURE

MILESTONE 4

In Vitro Evaluation

FUTURE

MILESTONE 5

Lead Optimization for Selected Series

FUTURE

MILESTONE 6

In Vivo Evaluation in Disease Model

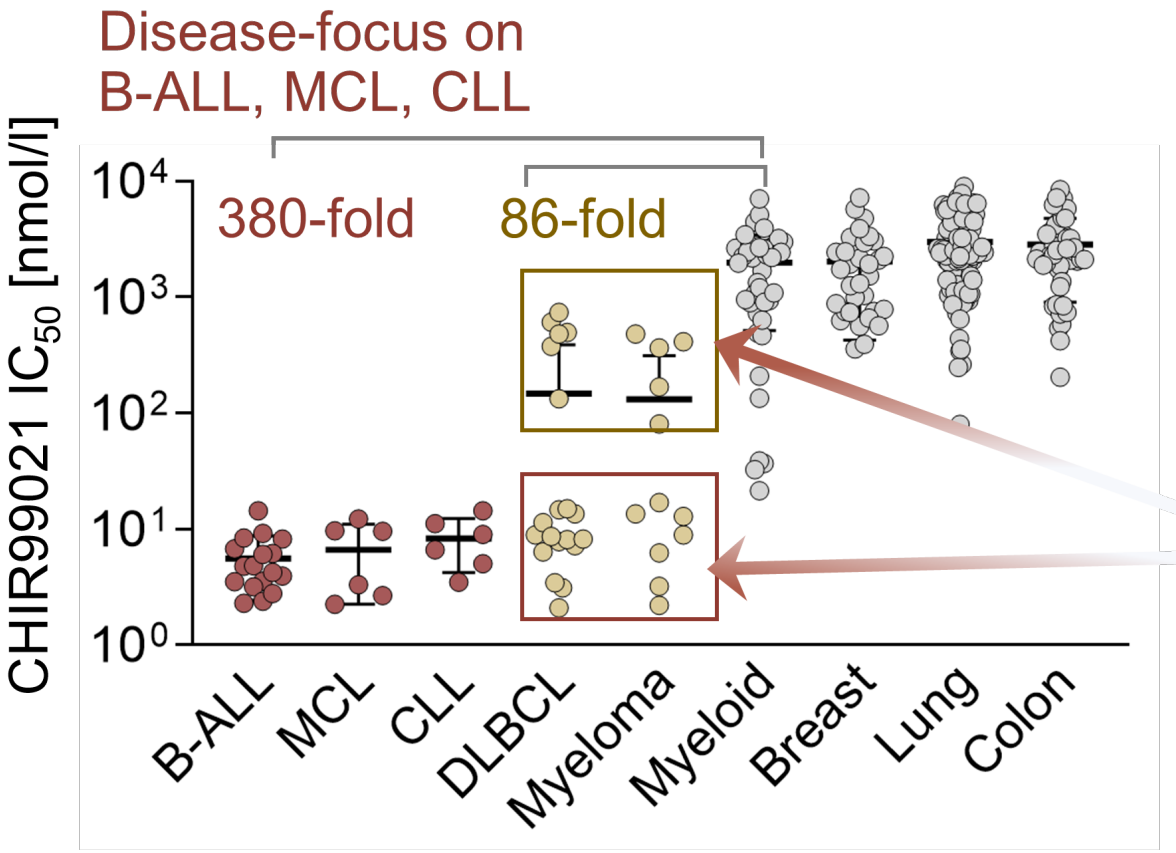
FUTURE

2024 Accelerator Award Winner

Proposed Milestones for Full Blavatnik Award ($300,000)

Appendix

Refined market analysis for GSK3β-inhibitors: Prioritize patients with B-ALL, CLL and MCL



Disease focus:

- *MYC*-translocations do not occur in B-ALL, MCL, CLL
- Unique sensitivity of B-ALL, MCL, CLL to GSK3β-inhibition.
- Bi-modal distribution of other B-cell lymphomas based on presence or absence of *MYC*-translocation.

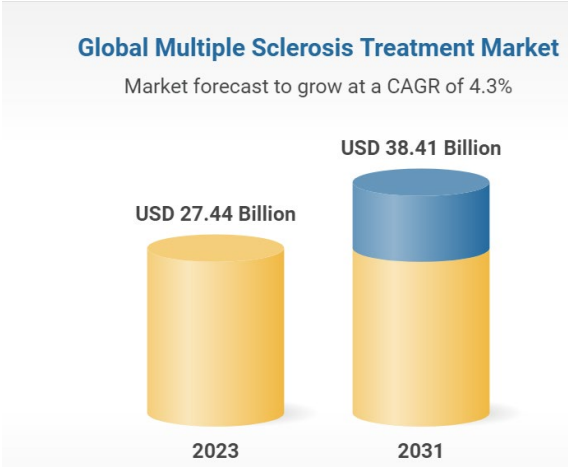
- We expect that patients with B-ALL, MCL, CLL will benefit the most.
- ~400,000 patients in the US

Multiple Sclerosis Approved Therapies Disease Landscape

Drug	Company	Target	Phase
Gilenya	NOVARTIS	sphingosine-1-phosphate receptor modulator	Approved
Aubagio	sanofi	Pyrimidine synthesis inhibitor	Approved
Copaxone	sanofi	Glatiramer acetate	Generic
Tecfidera	Biogen	Dimethyl fumarate	Generic
Plegridy	Biogen	Long-acting PEGylated interferon	Approved
Lemtrada	sanofi	Anti-CD52 mAB	Approved
Ocrevus	Biogen	Anti-CD20 monoclonal antibody	Approved
Cladribine (Mavenclad)	EMD SERONO	JAK1 and TYK2 inhibitor	Approved
Mayzent (Siponimod, BAF312	NOVARTIS	S1P receptor agonist	Approved
Vumerity	Biogen	Prodrug of monomethyl fumarate	Approved
Zeposia (Zeposia)	Bristol Myers Squibb	SP1 receptor agonist	Approved
Kysimpta (ofatumumab)	NOVARTIS	Anti-CD20 mAb	Approved
Povory (ponesimod)	janssen	S1P receptor agonist	Approved
Briumvi (Ublituximab)	TG Therapeutics	Anti-CD20 mAb	Approved

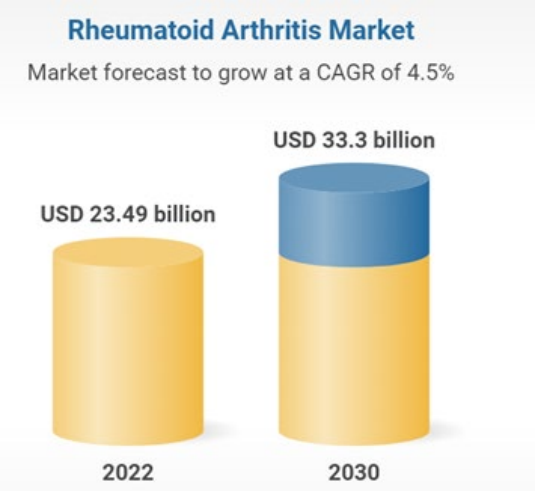
Multiple Sclerosis In Development Therapies Disease Landscape

Drug	Company	Target	Phase
Masitinib	AB SCIENCE	c-Kit inhibitor	II
Fenebrutinib	Genentech	Irreversible BTK inhibitor	II
Evobrutinib	EMD SERONO	BTK inhibitor	III
ATX-MS-1467/ M2736	EMD SERONO	Immune-tolerizing agent	II
MN-166 Ibudilast	MEDICINOVA	IL-1 Beta antagonist, IL-6 inhibitor, TNF-alpha inhibitor	II
BIIB091	Biogen	BTK inhibitor	I
FREQ-162	FREQUENCY THERAPEUTICS	Against novel remyelination target	Pre-Clinical



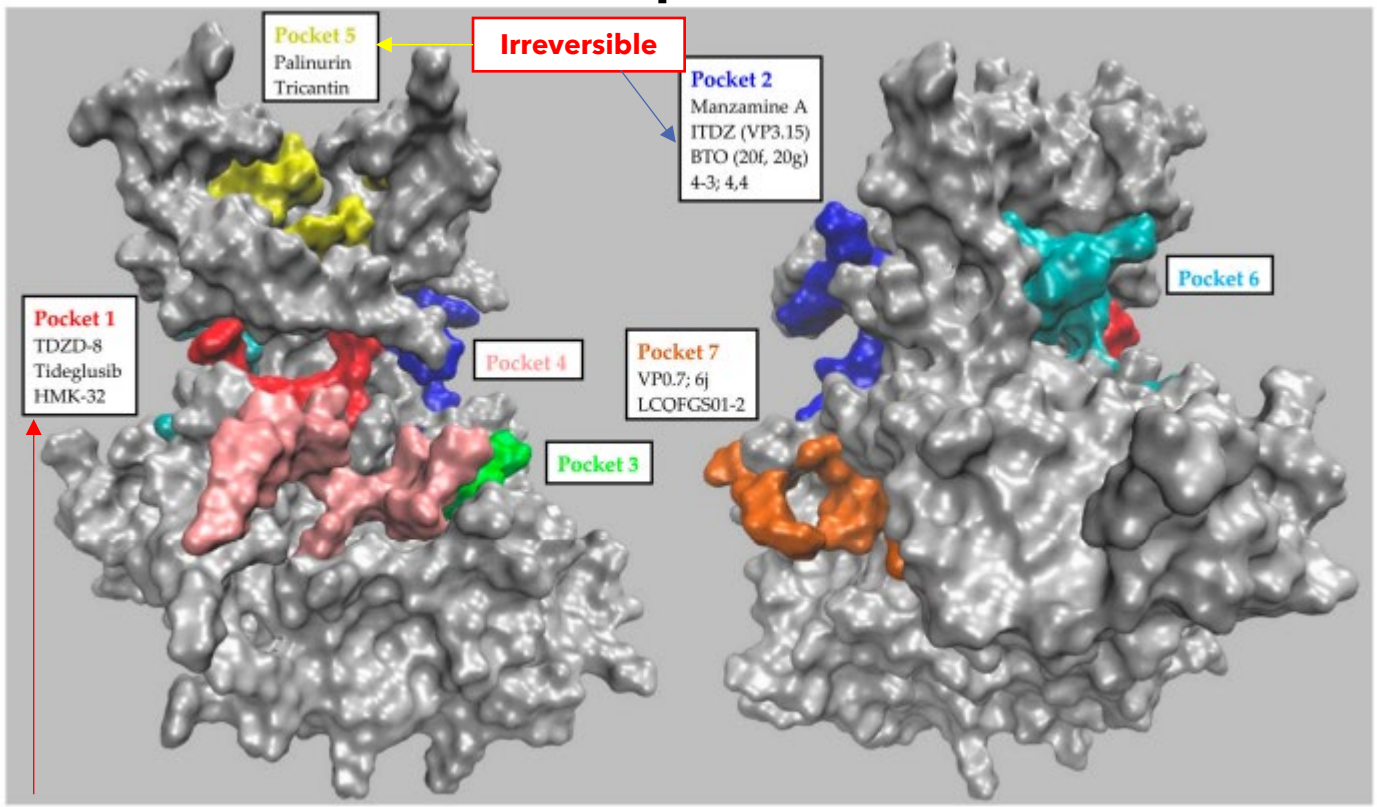
Oral Drug Rheumatoid Arthritis Landscape

Drug	Company	Target	Phase
Xeljanz (tofacitinib)	Pfizer	JAKi	Approved
Olumiant (baricitinib)	Lilly	JAKi (1/2)	Approved
Rinvoq (upadacitinib)	abbvie	Selective JAKi	Approved
PF-06650833	Pfizer	IRAK4 inhibitor	II
PF-06651600	Pfizer	JAK3/TEC inhibitor	II
Piclidenoson (CF101)	CANFITE BioPharma Ltd	A3 adenosine receptor agonist	III
AZD9567	AstraZeneca	SGRM	II
Dazodialibep	HORIZON	CD40L antagonist	II
KT-474	sanofi	IRAK4 degrader	I
BAY1830839	BAYER	IRAK4 inhibitor	I



Previous Strategy to Develop Specific GSK3B Inhibitors is Reversible Allosteric Binding to non-ATP pockets

Binding Sites of Previous GSK3B Inhibitors In Development



Low Selectivity, lead to off target effects

- The ATP Pocket (Pocket 1) homology is too similar between GSK3A and B: **Need to bind to GSK3B Specific Pockets** to limit off-target effects
- GSK3B implicated in other physiological pathways therefore inhibitor needs to be **reversible** to limit adverse effects
- Other Pockets have not been fully investigated for selectivity between GSK3A/B
- Its possible that more pockets exist

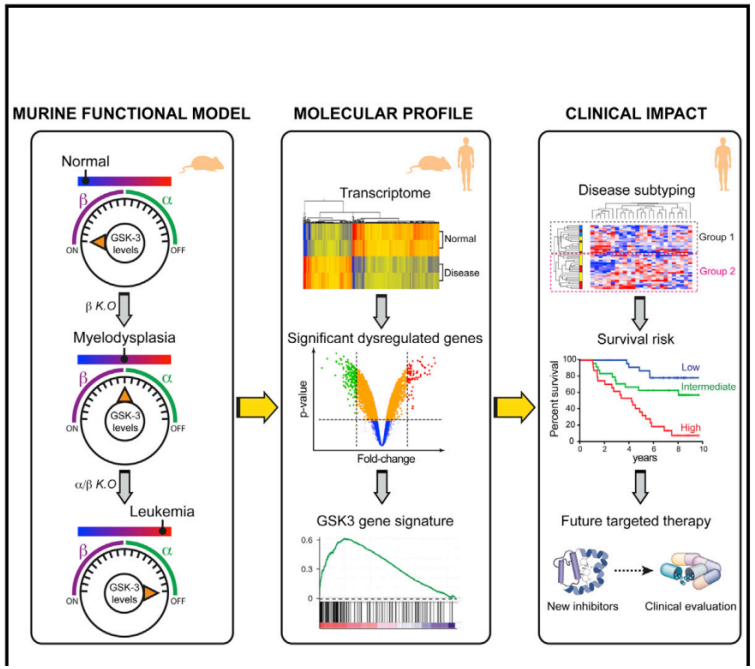
Rationale for GSK3B-selectivity:
GSK3A has undesired effects on myelopoiesis and the CNS

Article

Cancer Cell

GSK3 Deficiencies in Hematopoietic Stem Cells Initiate Pre-neoplastic State that Is Predictive of Clinical Outcomes of Human Acute Leukemia

Graphical Abstract



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In Brief

Guezguez et al. show that progressive removal of glycogen synthase kinase-3 (GSK-3) signaling by *Gsk3b* allelic deletion results in an MDS state that, when combined with *Gsk3a* deletion, leads to AML. A molecular signature derived from *Gsk3b*-null cells has prognostic potential for MDS patients.

Feasibility of HTS for GSK3B-selective compounds:
The structural basis for paralog-selective HTS has been solved

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

Exploiting an Asp-Glu "switch" in glycogen synthase kinase 3 to design paralog-selective inhibitors for use in acute myeloid leukemia

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Most Frequent B-Cell Malignancies

B-Cell Malignancy	Number of People Diagnosed in USA	% of People experiencing Relapsed/refractory B-Cell Malignancy
Chronic Lymphocytic Leukemia (CLL)	20,700	>20%
Hairy Cell Leukemia (HCL)	6,000	30-40%
Mantle Cell Lymphoma (MCL)	20,000	74%
Diffuse Large B-Cell Lymphoma (DLBCL)	80,000	30-40%
Follicular Lymphoma (FL)	15,000	20%
B-Cell Acute Lymphoblastic Leukemia (ALL)	80,000	20%