PROTAC THERAPY IN THE T-CELL MALIGNANCIES (*PROTEOLYSIS-TARGETING CHIMERAS*)

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T-Cell Lymphomas: Finally, Vision and Mission!
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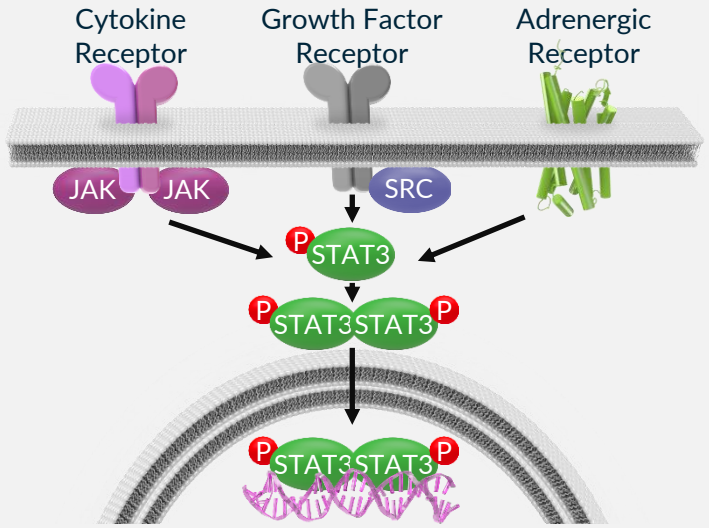
Bologna

PROTAC Therapy in the T-Cell Malignancies (*Proteolysis-Targeting Chimeras*)

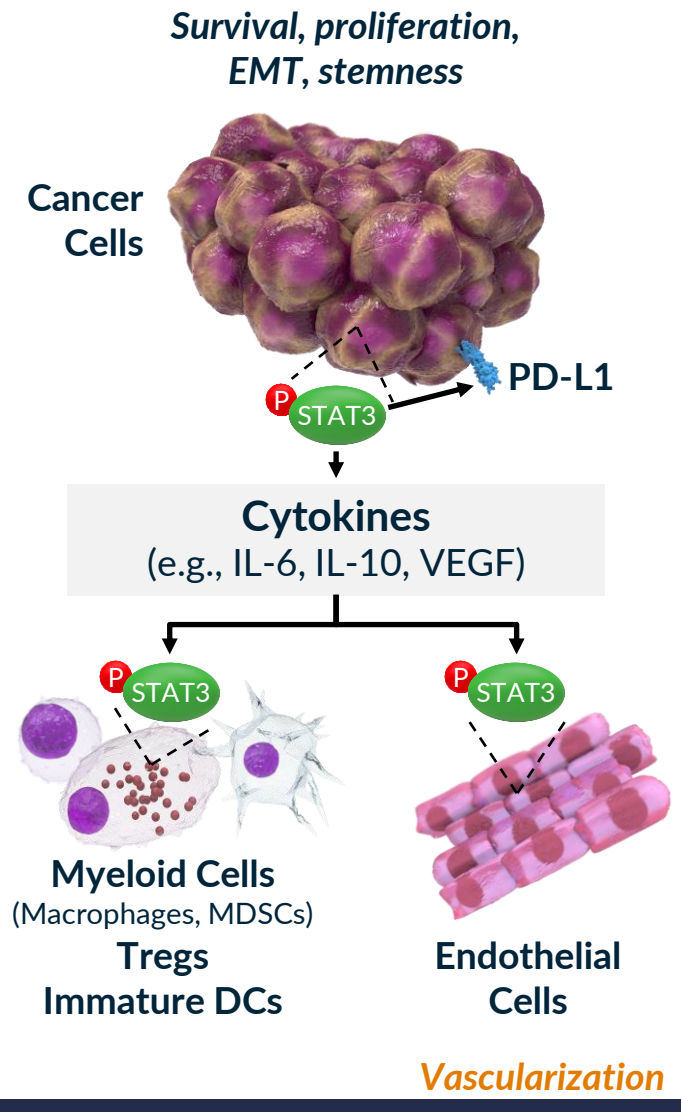
- Targeting STAT3 in PTCL: The First Foray of a PROTAC in the Disease
- Why Target STAT3 in PTCL?
- What is a PROTAC and Just How Does it Work?
- Preclinical Data with a STAT3 Targeted Protein Degrader
- The First Clinical Trial Design of KT-333, a STAT3 TPD
- Other Relevant Targets: MDM2
- Conclusion

STAT3 Has Unique Tumor Cell Intrinsic and Extrinsic Mechanisms

STAT3 as a Target



- High degree of validation of JAK-STAT pathway in oncology and immuno-oncology supported by >25k publications
- Traditionally undrugged target
- First-in-class opportunity to address STAT3 driven pathology across large and diverse indications



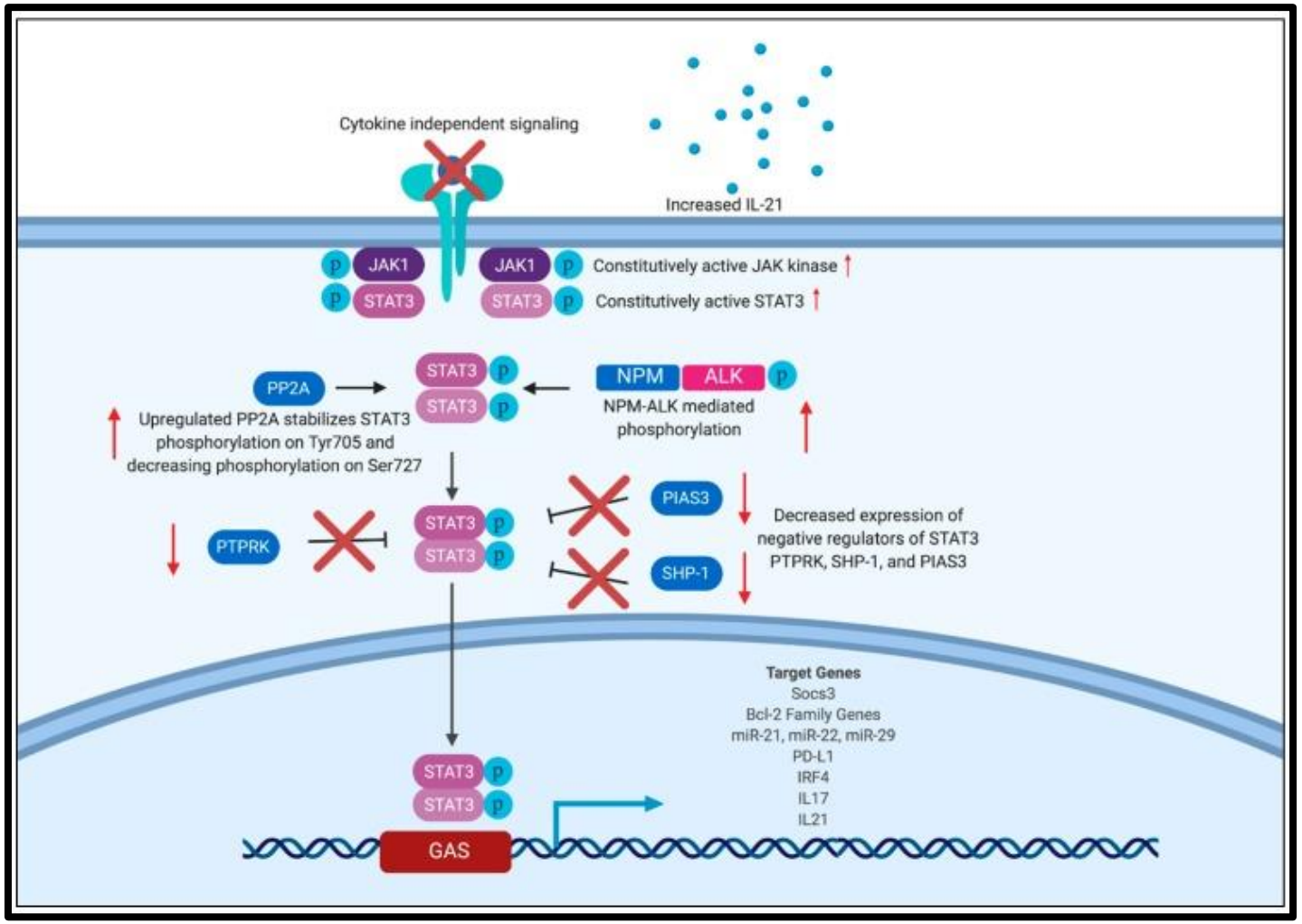
Tumor Cell Intrinsic

- Hyperactivation of STAT3 via either receptor signaling, or hotspot mutations promotes gene expression programs involved with survival, proliferation, stemness and metastasis of tumor cells
- Opportunities in STAT3-dependent malignancies (e.g., T cell malignancies, DLBCL, AML) and drug resistant tumors (e.g., TKI resistant oncogene-driven solid tumors)

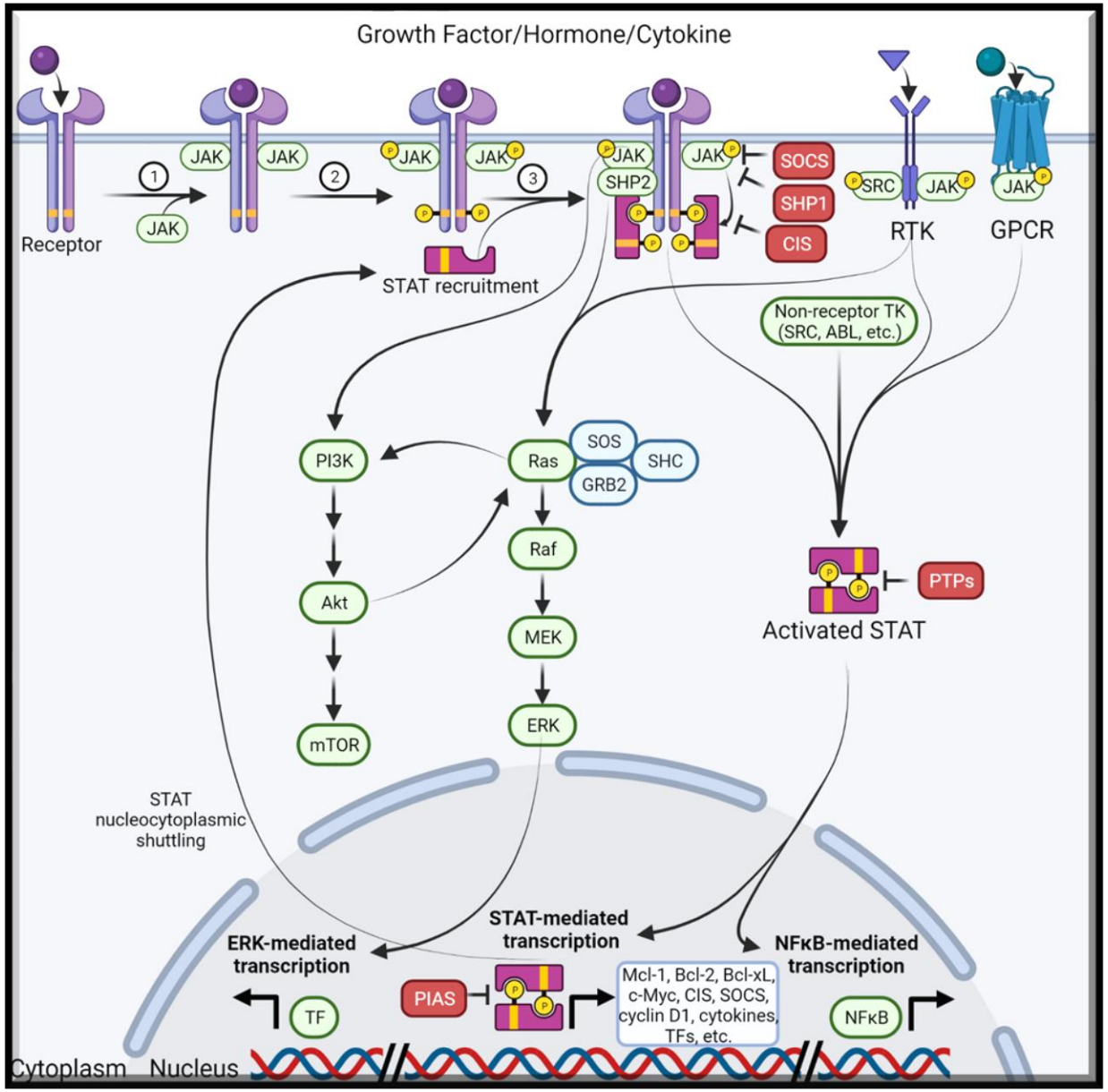
Tumor Cell Extrinsic

- STAT3 promotes the differentiation and activity of immunosuppressive and endothelial cells, resulting in an immunosuppressive tumor microenvironment.
- Opportunities in multiple heme and solid tumor indications that are not responsive to immune checkpoint inhibitors.

JANUS TYROSINE FAMILY OF KINASES/SIGNAL TRANSDUCE AND ACTIVATOR OF TRANSCRIPTION (JAK/STAT) IS COMMONLY DYSREGULATED ACROSS MANY TYPES OF T-CELL MALIGNANCY



- STAT3 is a transcription factor that mediates signal transduction through cytokine receptors (IL06R, IL-10 and IL-21)
- It enhances pro-survival signaling essential for T-cell expansion.
- When it becomes constitutively activated – that is, independent of the cytokine binding – it contributes to T-cell lymphomagenesis
- Previous efforts to target this pathway therapeutically have not been very successful

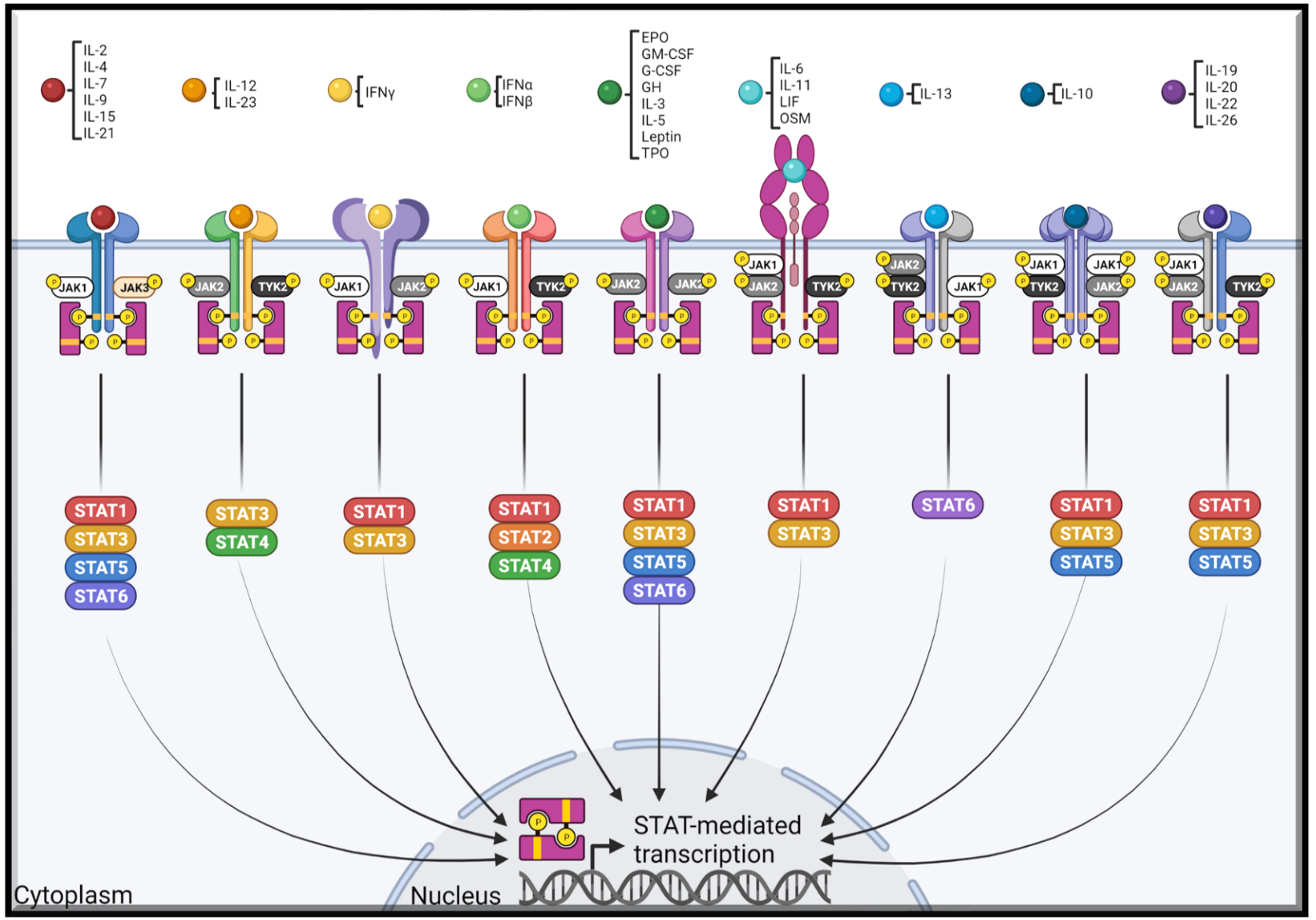


① After binding of a cytokine to its receptor JAK-family kinases are recruited to the cytosolic domain of the receptor and facilitate auto- and/or transphosphorylation of tyrosine residues on the JAK proteins and the receptor

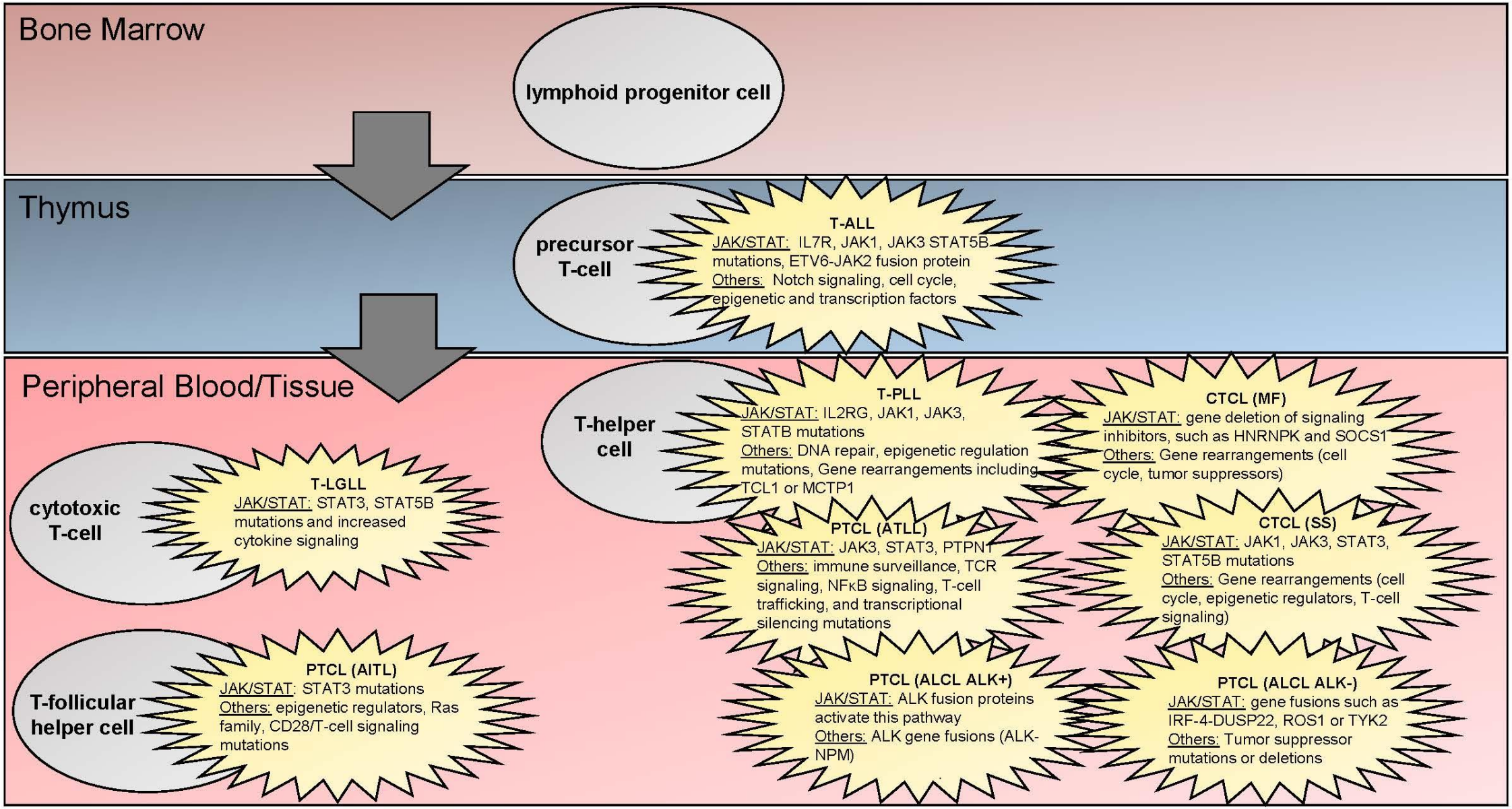
② Specific STAT subunits, dependent on the receptor-JAK combination, are recruited, docked on the phosphorylated receptor tyrosine residues, and activated by a series of phosphorylation events

③ Activated STAT is translocated to the nucleus and participates in STAT-mediated transcription whilst promoting NF-κB transcription.

- Receptor tyrosine kinases (RTKs), non-receptor TKs, and G-protein coupled receptors (GPCRs) can also activate STAT.
- JAK activation leads to crosstalk with the PI3K-Akt-mTOR and Ras-Raf-MEK-ERK pathways.
- Endogenous JAK-STAT inhibition also includes the activity of PTPs like SHP1, which inhibit JAK-STAT proteins via dephosphorylation.



- A **diverse repertoire of cytokines bind** to their cognate receptors and lead to JAK/STAT3 activation
- There are **subtle differences in the JAK and STAT signaling components** of each cytokine pathway
- **STAT3 is common component** across most of the cytokine mediated pathways



Alterations in JAK/STAT Signaling as a Function of T-Cell Ontogeny in Normal and Malignant Cells

Constitutive Activation of STAT3 is Common Across PTCL Subtypes

Significant Variation is Pathway Dysregulation

Disease	Frequency (pSTAT3 positive)
Across PTCL (n=169)	38%
ALK(+) ALCL	93% (STAT3 is target for NPN-ALK)
ALK(-) ALCL	57%
AITL	29%
ATLL	43% (improved survival)
LGL	More common in CD8+, and correlate with shorter TTF and AIHA and RA
EATL	16% (most commonly mutated signaling pathway)

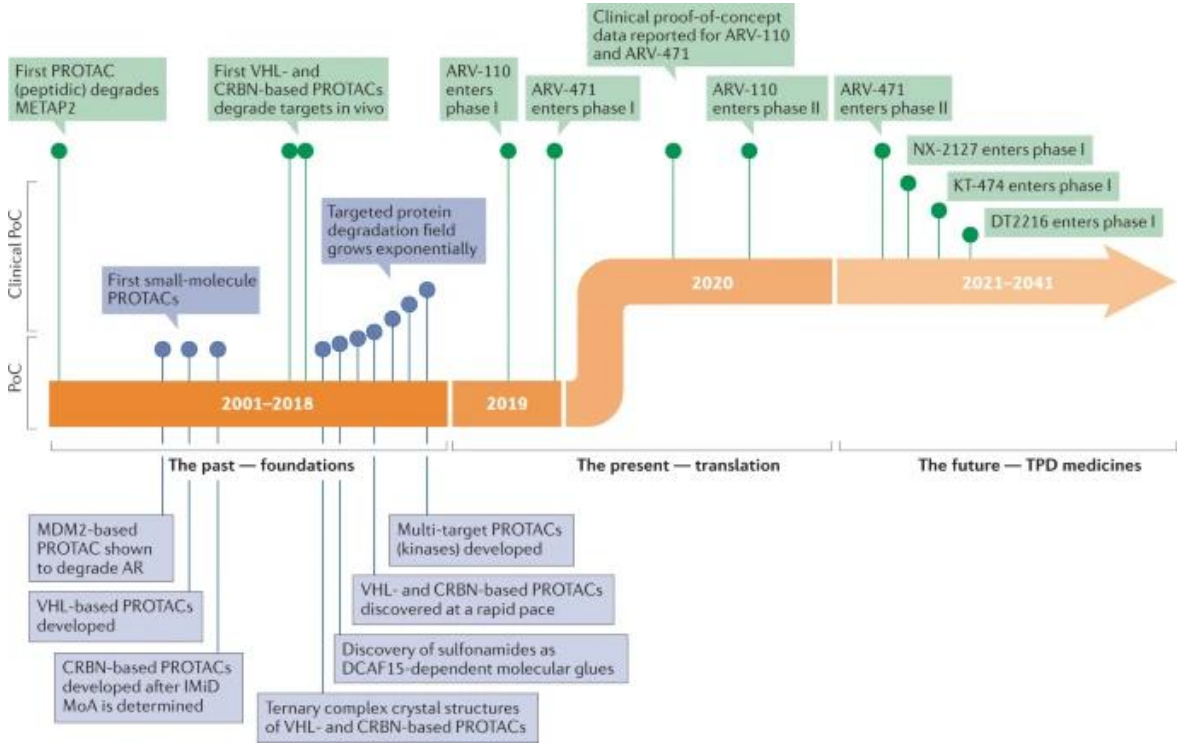
PROTAC Therapies……From the Beginning

PROTAC = Proteolysis targeting chimera

The concept is drawn from viruses and plants which have evolved sophisticated strategies to hijack the ubiquitin-proteasome system for its own survival

For example, the E6 protein of human papillomavirus type 16 (HPV-16) and type 18 (HPV-18) recruits the human E3 ligase, ubiquitin–protein ligase E3A (also known as E6AP) to ubiquitylate p53, resulting in loss of tumor suppressor functions.

Human immunodeficiency virus 1 (HIV-1) deploys Vpr and Vpx, to recruit DDB1 and CUL4-associated factor 1 (DCAF1) to target several different human proteins, including DNA repair proteins, for ubiquitylation (makes cell error prone)



Therapeutic significance conceived around 2000

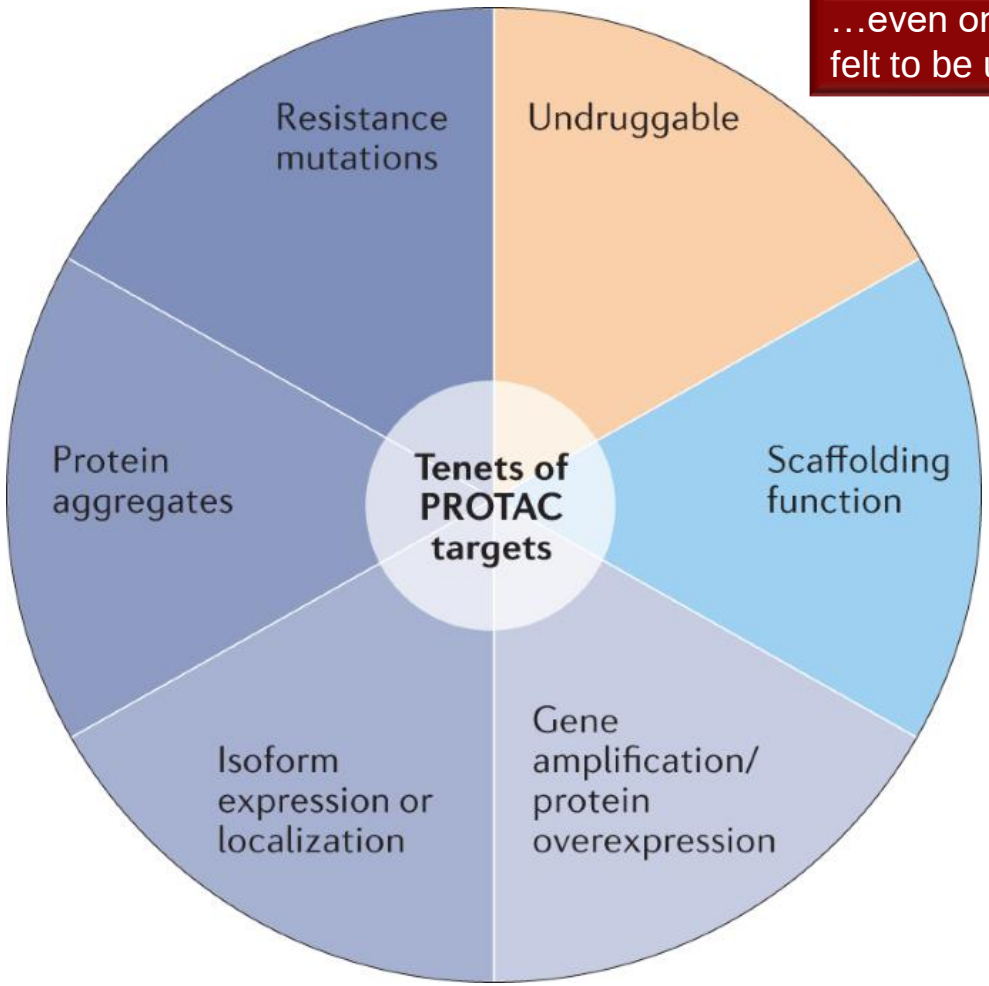
Arvinas was first to clinic with AR degraders (ARV110) and ER degrader (ARV471)

VIRTUALLY ANY PROTEIN CAN BE TARGETED…..

….because every protein in the cell needs to come and go…..and the **E3 ligase is the major pathway to elimination of intracellular protein.**

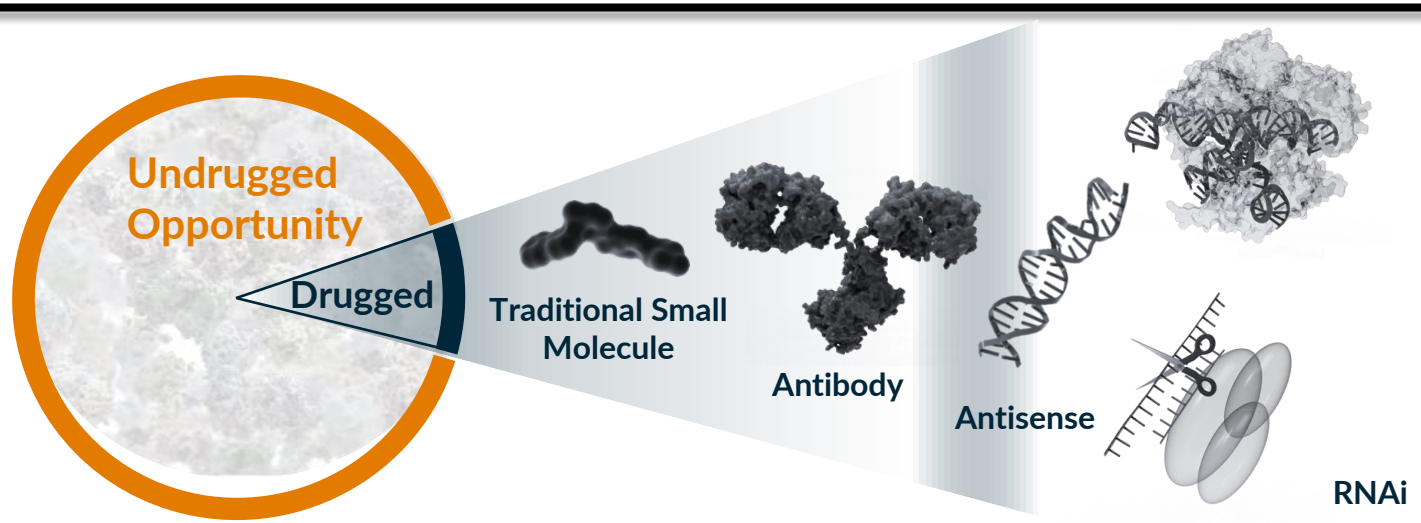
- Small molecule binds to E3 and target protein to effect its degradation
- Small Molecule only needs to "**weakly**" bind to protein and does have to inhibit its function
- Highly potent and specific hence small amount of drug needed
- **Highly specific**
- Can **create genetic–like knock-down effects**
- Advantages of small molecule development: Route of administration, manufacturing

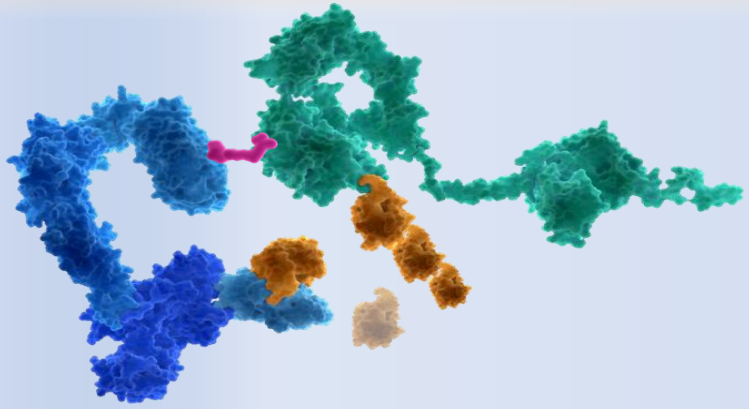
…even ones previously felt to be undruggable



PROTAC Therapies Offer the Prospect of Expanding the Druggable Proteome with Targeted Protein Degradation (TPD)

Presently available therapeutic modalities can **only drug up to 20%** of proteome





PROTAC Therapies can **expand the drugged proteome** with Targeted Protein Degradation (TPD)

Targeted Protein Degradation

Co-opting a Naturally Occurring Process to Regulate Protein Levels

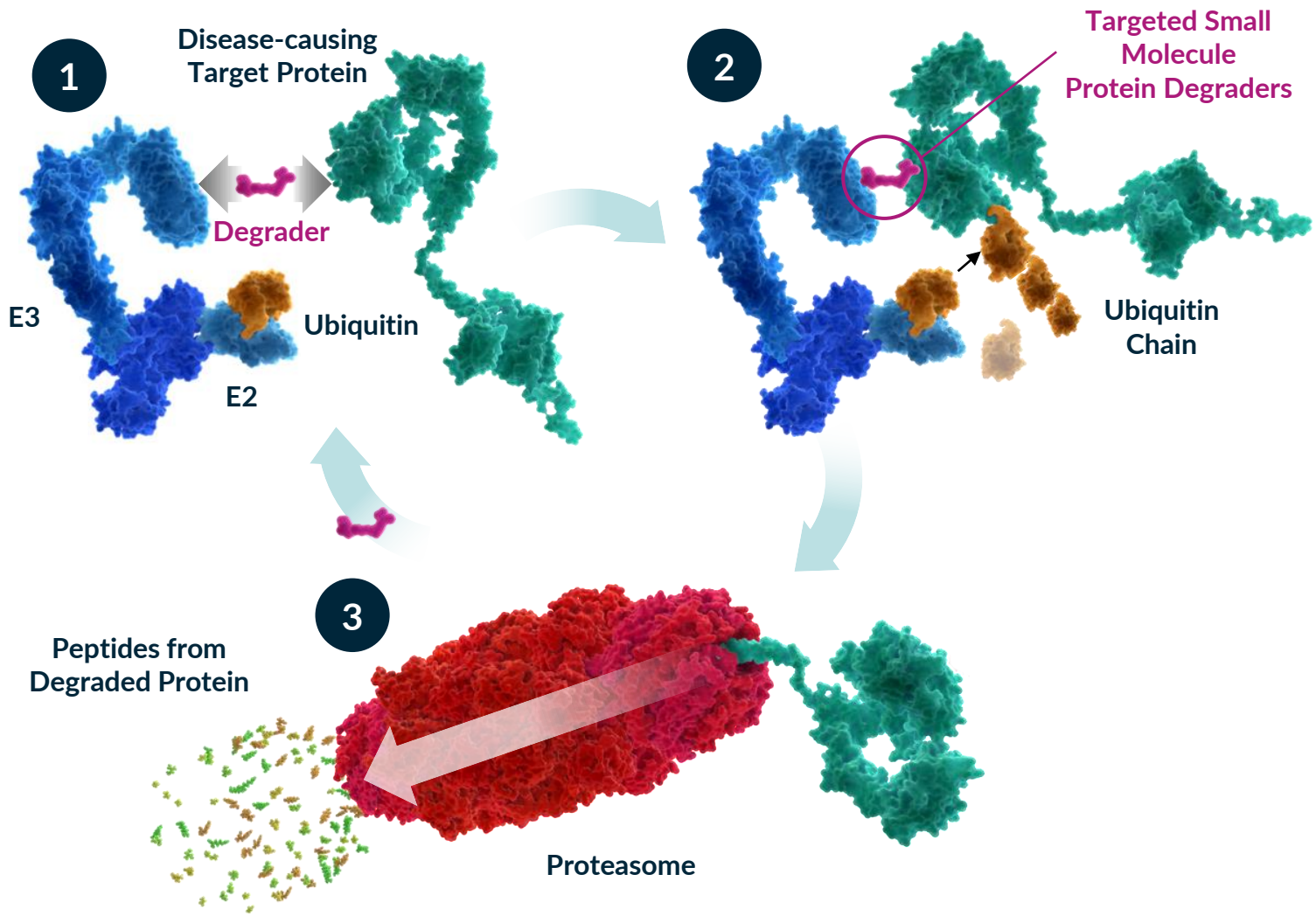
1. E3 ligase recognizes protein
2. Ubiquitin chain transferred
3. Protein is marked for elimination

KYMERA

Broad Opportunity
Only Binding Site Required

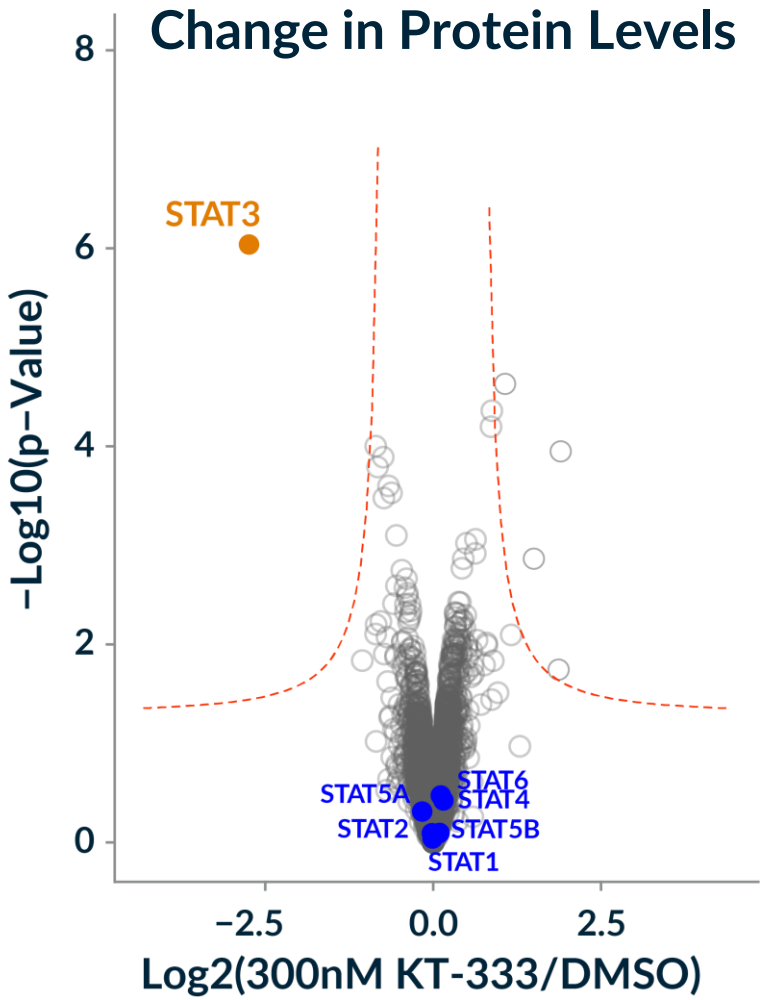
Efficient
Catalytic

Prolonged Impact
Targeted Protein Degradation



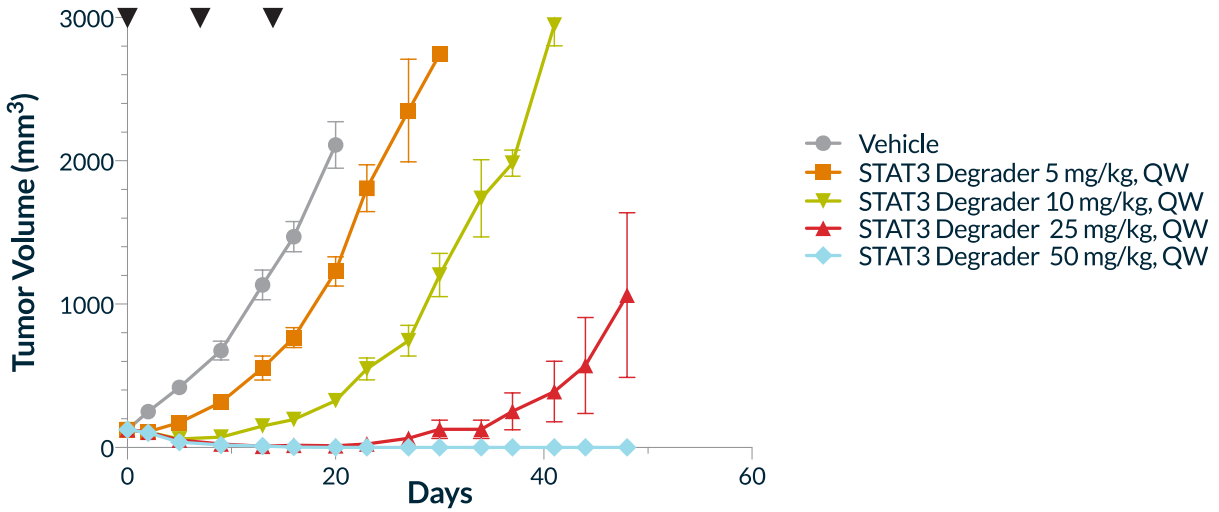
KT-333 Demonstrates Highly Selective Degradation of STAT3

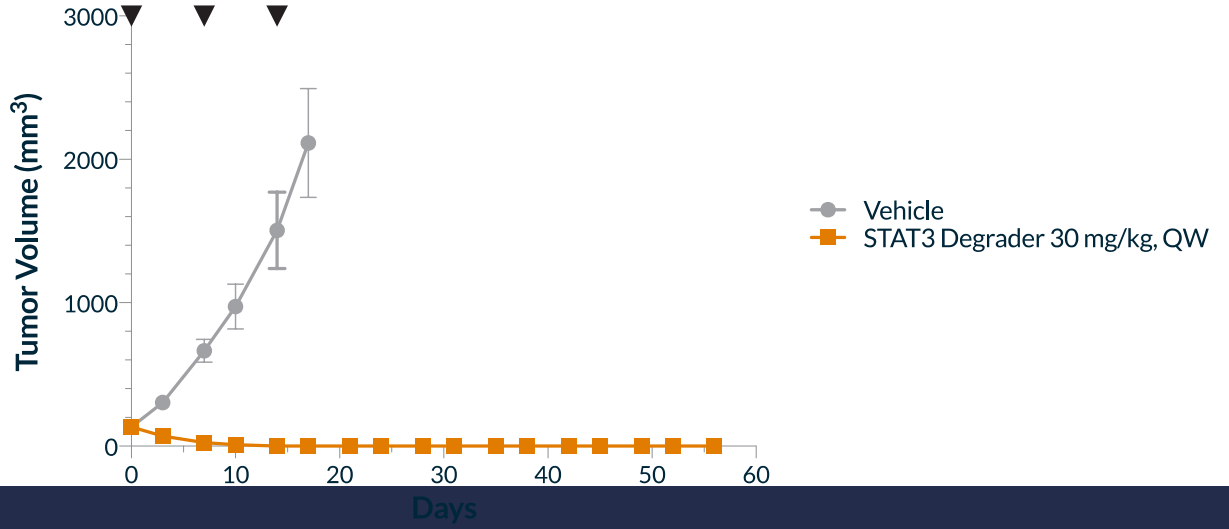
- Deep mass spectrometry-based proteomics to assess STAT3 selectivity performed
- In hPBMC and SU-DHL-1 cancer line (shown), treatment with KT-333 degrader led to selective degradation of only STAT3 protein



Full and Durable Regressions Across Multiple *in vivo* Preclinical Tumor Models

- Mice bearing STAT3-dependent ALK+ ALCL SU-DHL-1 (above) and STAT3-driven ALK+ ALCL xenograft model SUP-M2 (below) tumors dosed with STAT3 degrader
- Dose and degradation dependent tumor growth inhibition observed with once-a-week IV dosing
- 30 mg/kg sufficient to drive full tumor regression that was durable for multiple weeks after the last dose

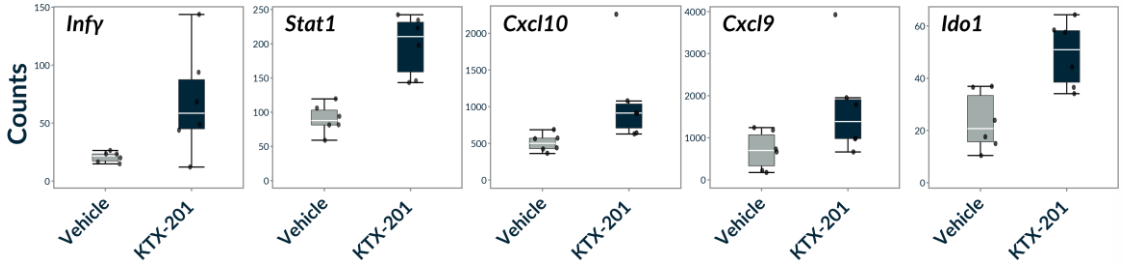




STAT3 Degrader Sensitizes to PD-1 Blockade

Syngeneic Colon Carcinoma Model

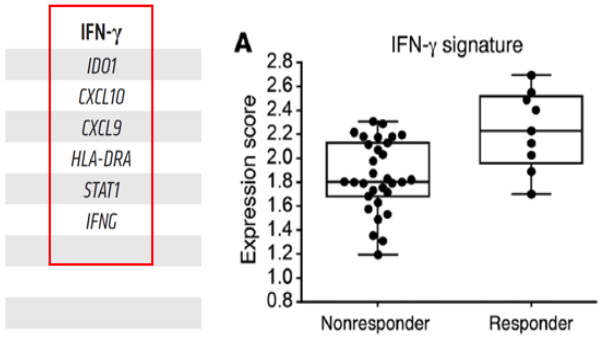
IFNγ-dependent Gene Signature Induced by STAT3 Degrader Monotherapy in CT-26 Tumors



CT-26: Veh or KTX-201 25 mg/kg q2D IP; n=6/grp; t = Day 11

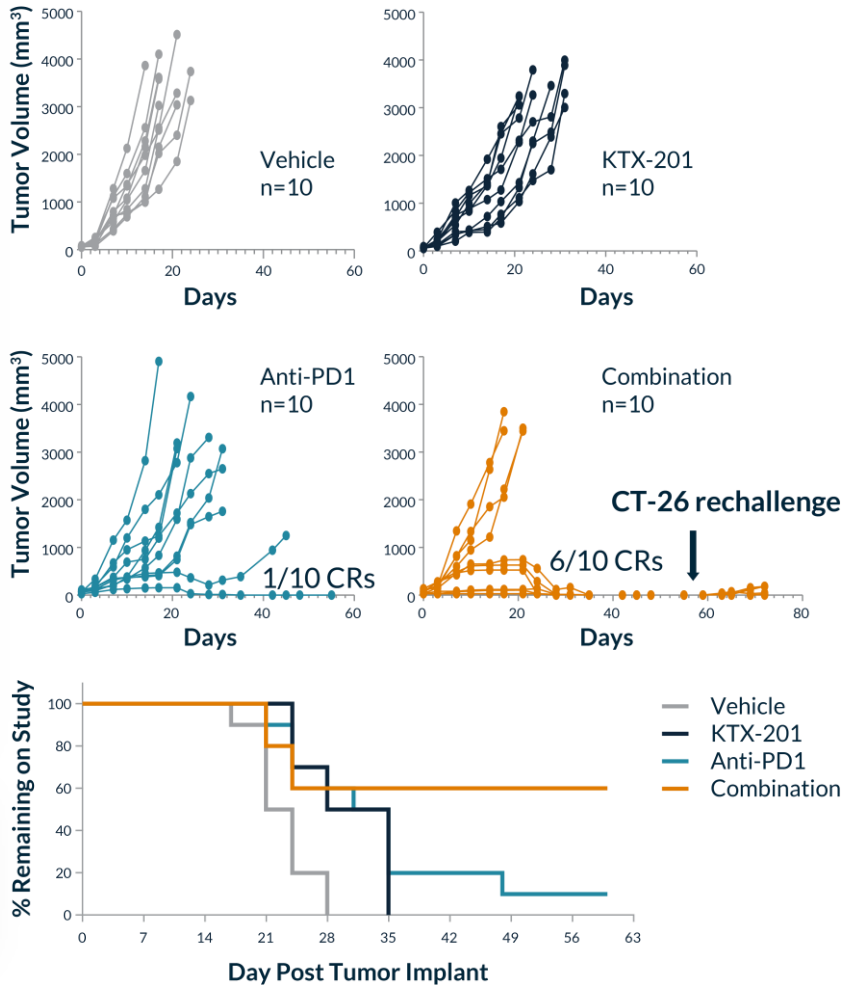
- STAT3 degradation remodels the CT-26 TME to be more immune-favorable with upregulation of anti-tumor immunity genes previously identified as predictors of clinical response to pembrolizumab

IFN-γ and Expanded Immune Gene Signature



- KTX-201 synergizes with anti-PD-1 leading to 60% complete responses in CT-26 model
- Complete responders reject tumor rechallenge demonstrating development of long-term immune memory
- Combination extends survival

STAT3 Degradation and Anti-PD-1 Synergy



KT-333: Clinical Study Design and Objectives

Key Eligibility Criteria:

R/R B-cell lymphoma

- ≥ 2 prior systemic regimens
- Ineligible or refused CAR-T or ASCT

Advanced solid tumors

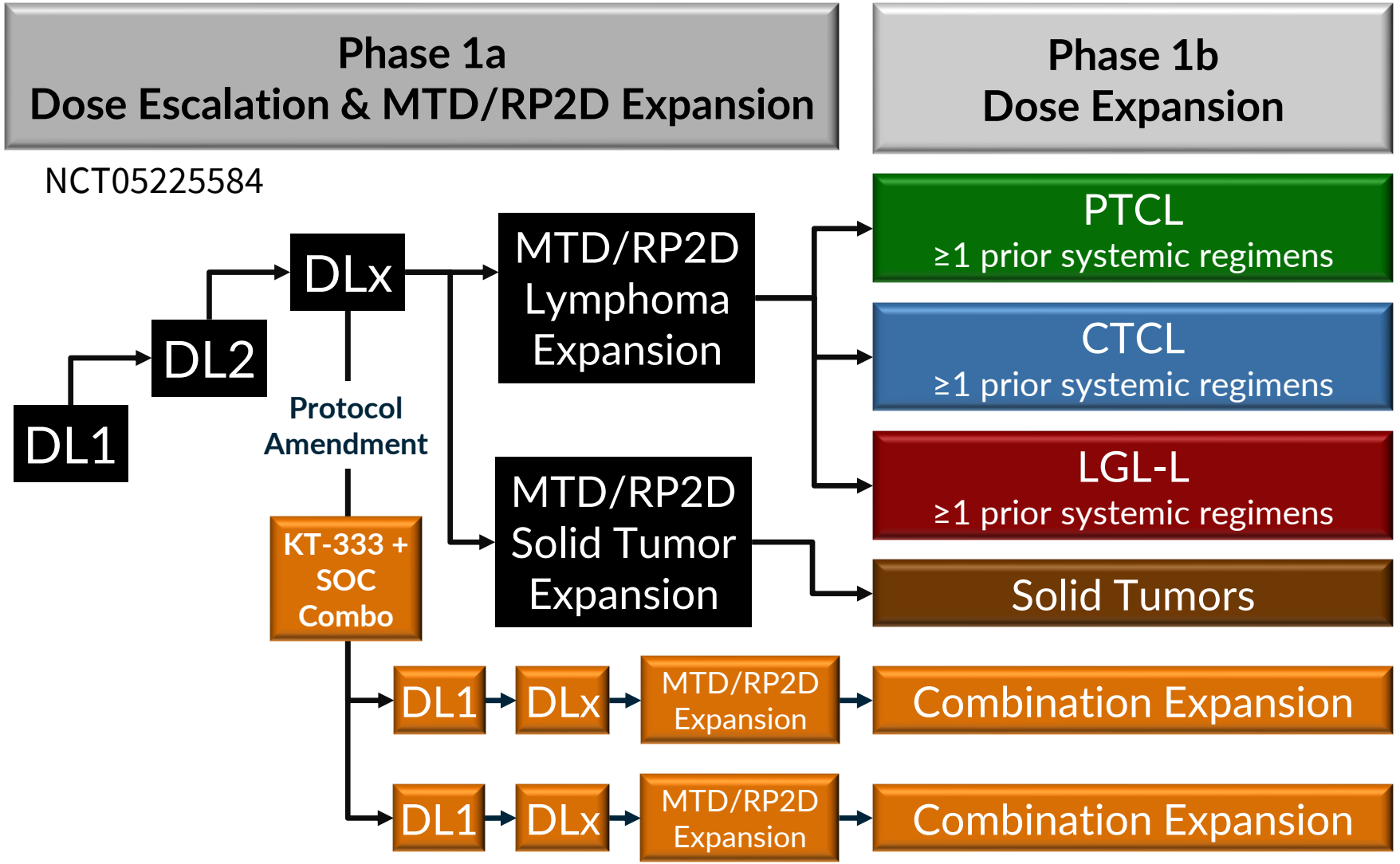
- ≥ 2 prior systemic regimens or no available SOC

Primary Objective:

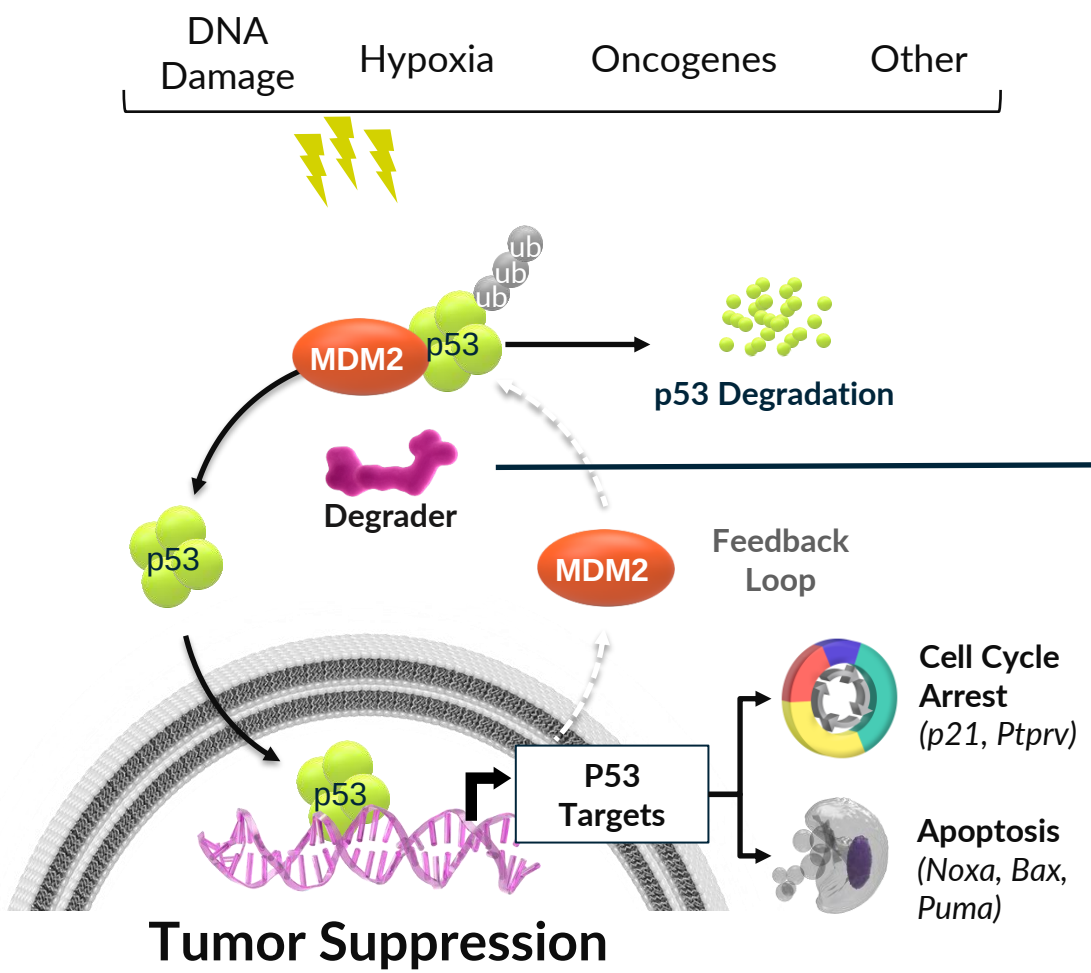
- To evaluate safety, PK/PD in PTCL, CTCL, LGL-L and solid tumors

Study Endpoints:

- Primary: Safety, tolerability, MTD/RP2D
- Secondary: PK, preliminary efficacy
- Exploratory: STAT3 knockdown and downstream effects in PBMC and tumor



MDM2 Degradation, Not Inhibition, Efficiently Restores p53



Clinical Validation

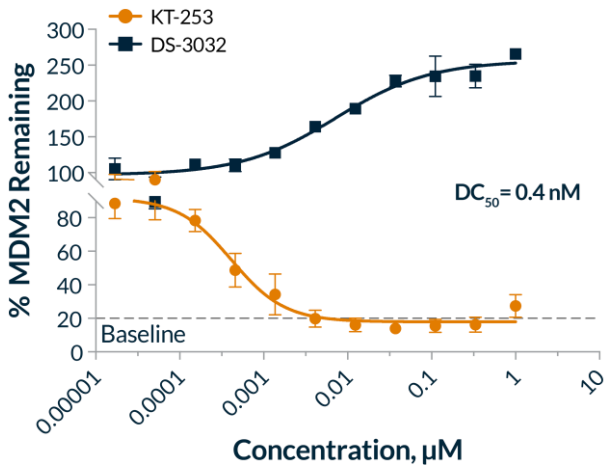
- **MDM2 small molecule inhibitors** of MDM2/p53 interaction **show activity in the clinic**..
- …**but they induce MDM2 feedback loop** resulting in limited impact on pathway

Degrader Advantage

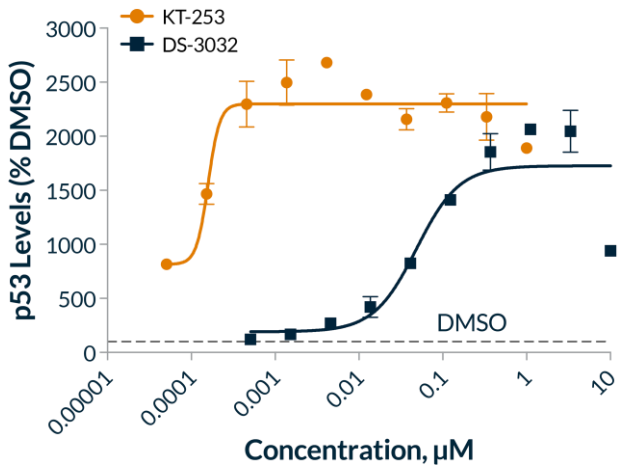
- **MDM2 degraders**, by removing the protein, **can overcome the p53-dependent feedback loop that upregulates MDM2**
- MDM2 degrader can induce an acute apoptotic response in tumor cells, **increasing efficacy and therapeutic index vs a small molecule inhibitor**

KT-253 - MDM-2 Degrader Development Candidate Appears Superior to MDM2/p53 Small Molecule Inhibitors

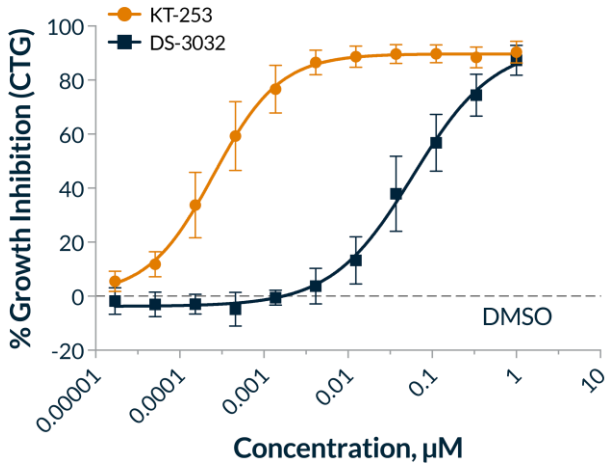
KT-253 is a potent MDM2 degrader



KT-253, unlike SMI's such as DS-3032, strongly stabilizes p53…



… which leads to superior tumor cell killing (pM range)



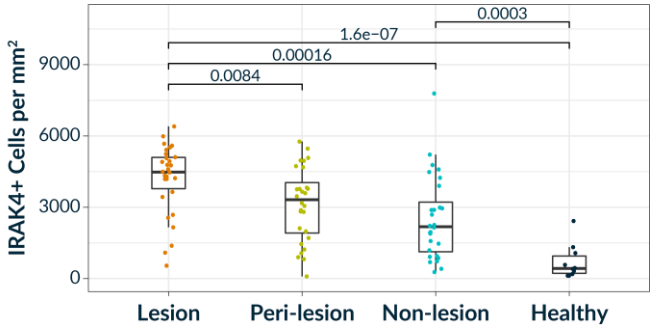
Compound	KT-253	DS-3032	RG7388	SAR405838	HDM201	AMG-232
Company	Kymera	Sankyo/Rain	Roche	Sanofi	Novartis	Amgen/Kartos
Clinical stage	IND enabling	Ph II / combo AML	Ph II / III	Paused	Ph I / II	Multiple Ph II; combo AML
RS4-11 IC_{50} (nM) (AML Cell Killing)	0.3	67	220	620	163	280
MDM2-HiBiT, DC_{50} (nM) (Degradation)	0.4	-	-	-	-	-

IRAK4 Protein Expression in Autoimmune Diseases

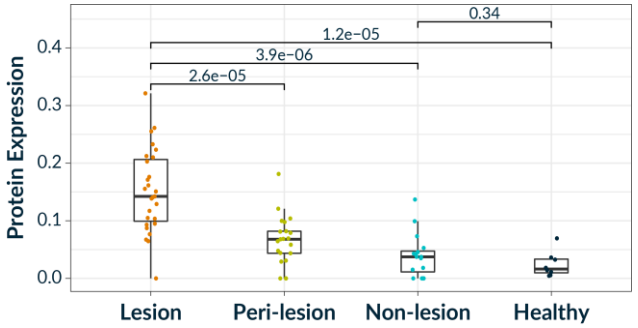
Upregulation in Skin of HS Patients Compared to Healthy Subjects

IRAK4 protein levels overexpressed in HS patient skin lesions

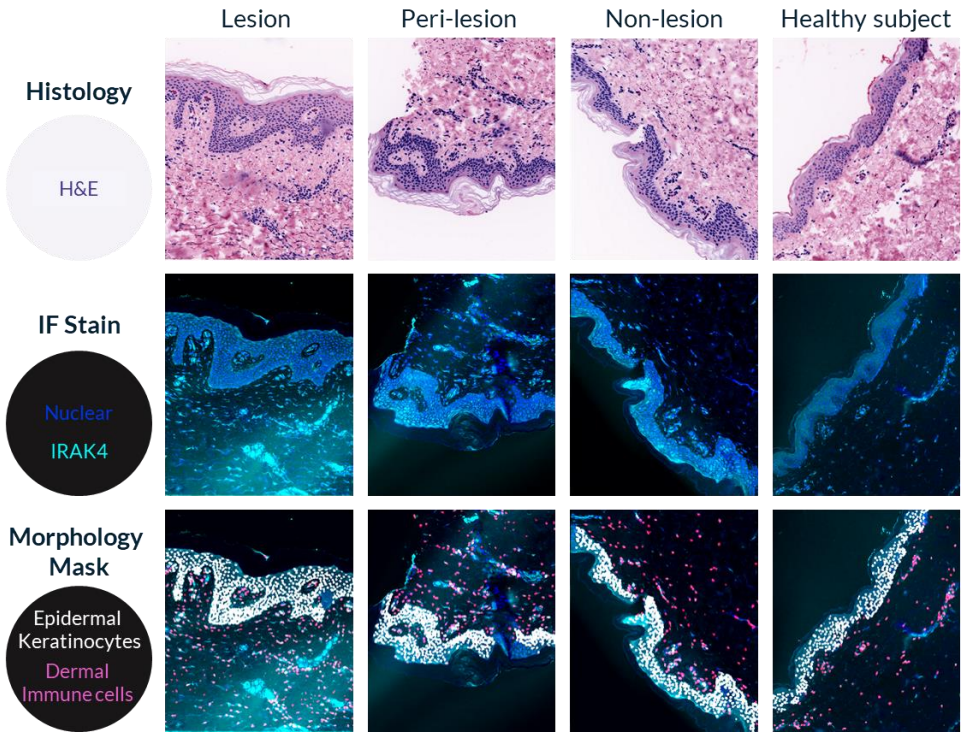
Immunofluorescence (IF)



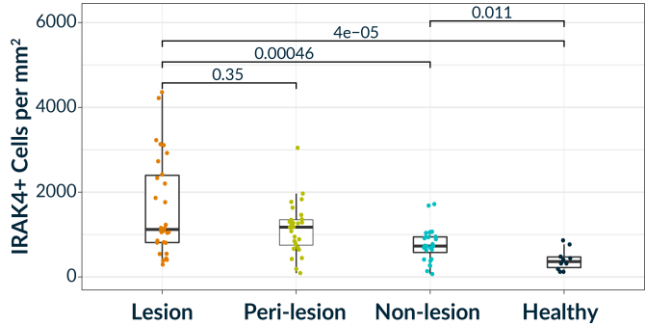
Mass Spectrometry (MS)



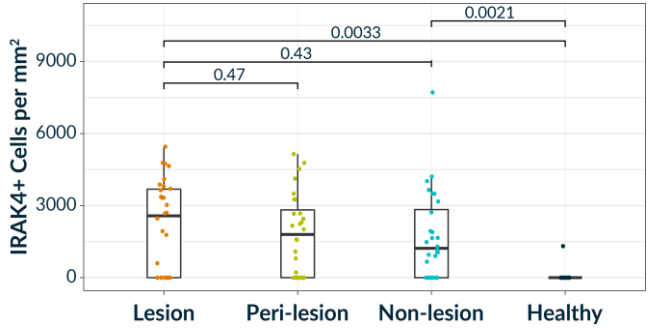
IRAK4 expression is upregulated in dermis and epidermis of HS patients relative to healthy subject skin



Dermal Immune Cells



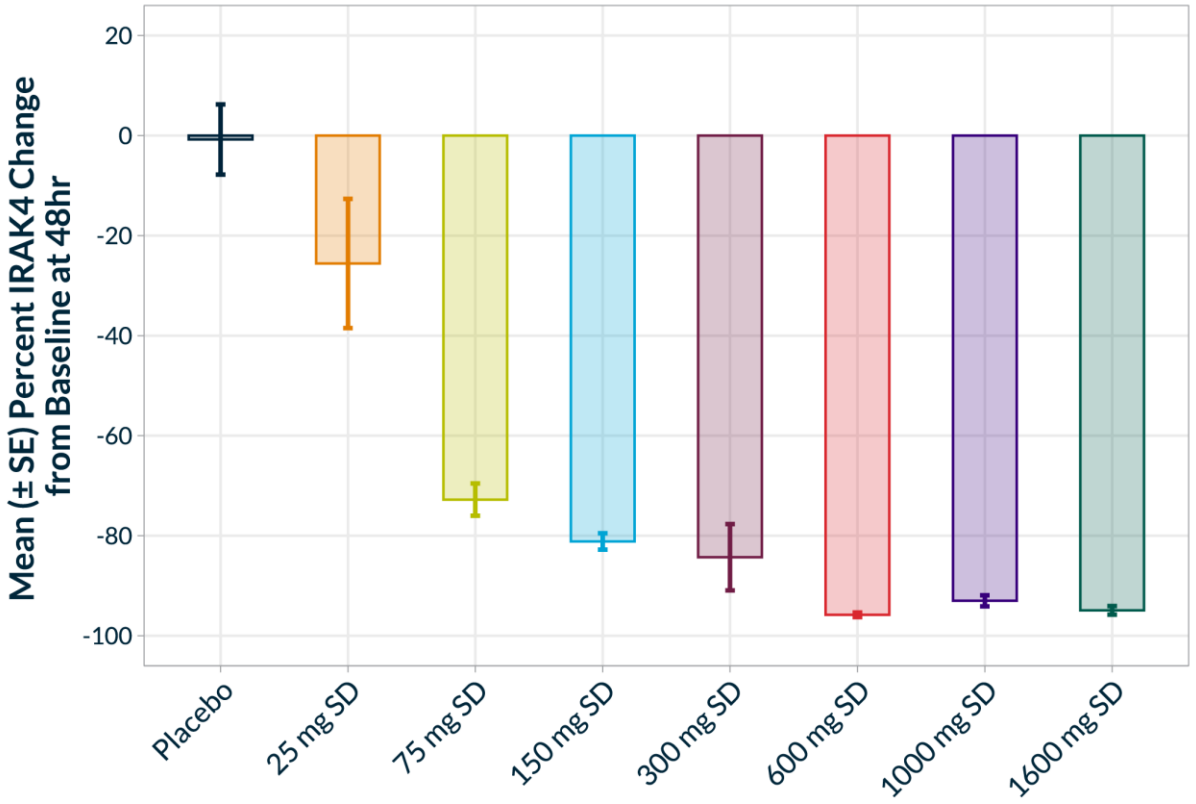
Epidermal Keratinocytes



Alavi et al., Society for Investigative Dermatology Annual Meeting, 2021

KT-474 Achieved >95% IRAK4 Degradation After Single Dose

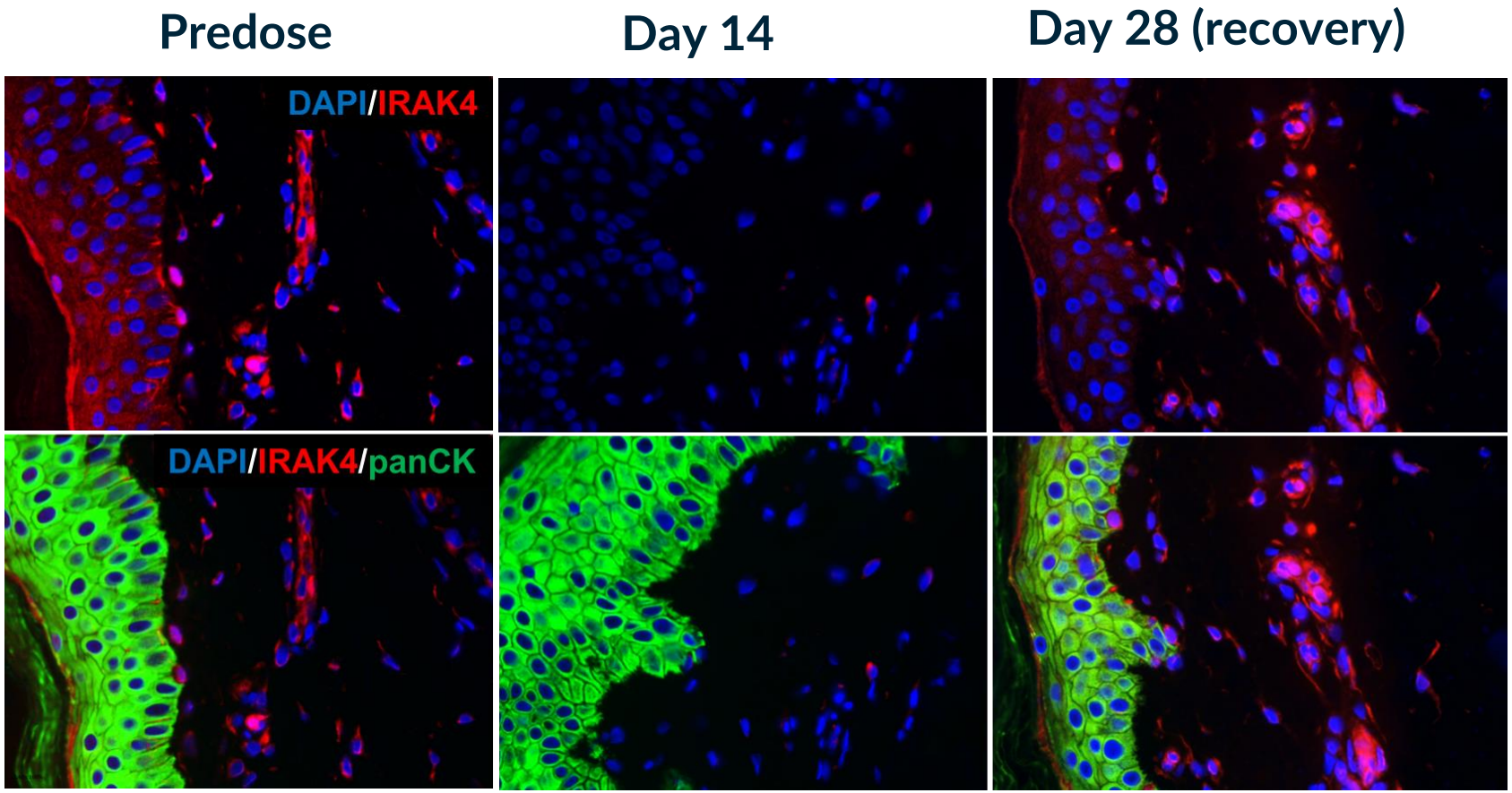
Percent IRAK4 Reduction in PBMC at 48 Hours Post-Dose Using Mass Spectrometry



	N	Mean IRAK4 Change	Median IRAK4 Change	p value
Placebo	13	-1%	-2%	--
25 mg	6	-26%	-39%	0.1
75 mg	6	-73%	-75%	<0.0001
150 mg	6	-81%	-82%	<0.0001
300 mg	6	-84%	-89%	<0.0001
600 mg	7	-96%	-96%	<0.0001
1000 mg	5	-93%	-94%	<0.0001
1600 mg	6	-95%	-95%	<0.0001

Substantial IRAK4 Degradation in Skin Observed in Dermis and Epidermis

IRAK4 = Red



Representative images from subject in 50 mg cohort

PROTAC Therapy in the T-Cell Malignancies
Conclusions

- PROTAC therapies, if they fulfil the hype, will be paradigm changing in the treatment of many diseases, not just cancer
- STAT3 certainly represents a reasonable unifying pathway to target, though its part of a complex network and may be not be sufficient alone
- Early phase studies in Hydradenitis suppurativa – an IRAK4 driven autoimmune disease of the skin – KT-474 selective degrades the intended target in patients
- An ongoing Phase 1 study of KT-333 dedicated to address the many subtypes of PTCL is underway and will be a pivotal breakthrough if reproducible signals can be demonstrated across STAT3 dysregulated PTCL
- Our laboratory is exploring synergistic combinations with KT-333

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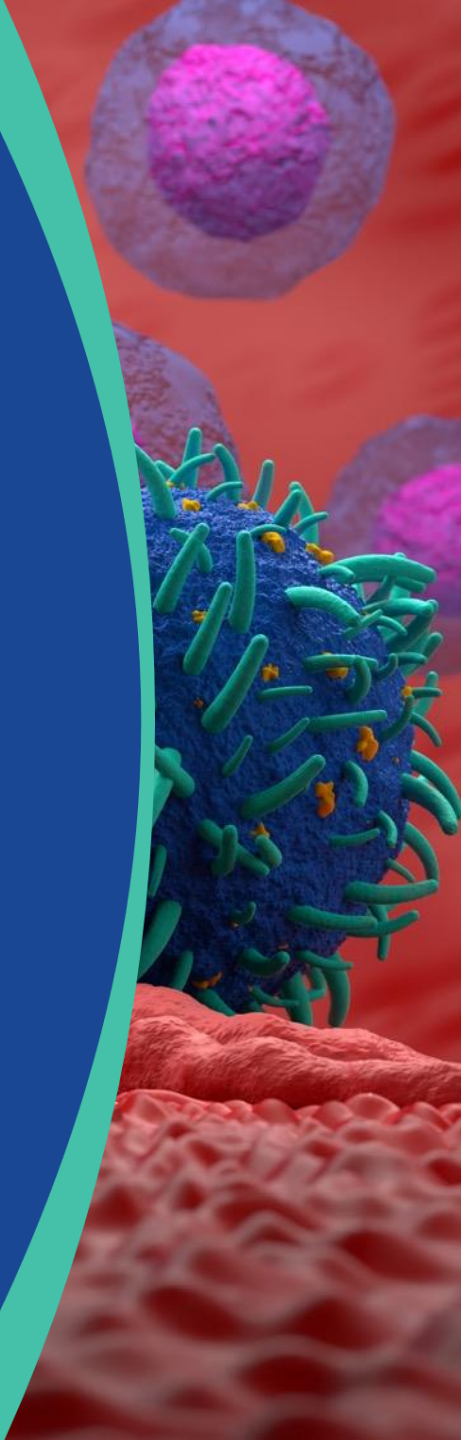

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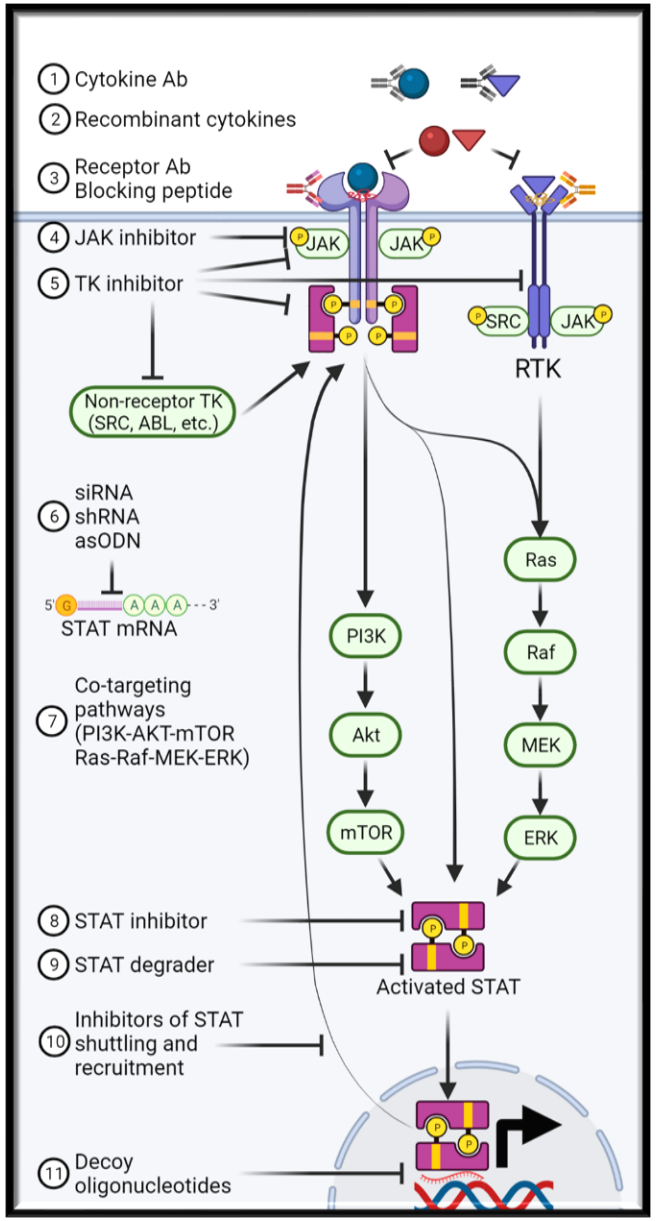
1 Cytokine Ab
2 Recombinant cytokines
3 Receptor Ab
Blocking peptide
4 JAK inhibitor
5 TK inhibitor
JAK
JAK
SRC
JAK
RTK
Non-receptor TK
(SRC, ABL, etc.)
6 siRNA
shRNA
asODN
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STAT mRNA
7 Co-targeting
pathways
(PI3K-AKT-mTOR
Ras-Raf-MEK-ERK)
PI3K
Akt
mTOR
Ras
Raf
MEK
ERK
8 STAT inhibitor
9 STAT degrader
Activated STAT
10 Inhibitors of STAT
shuttling and
recruitment
11 Decoy
oligonucleotides