Restoration of the apoptotic threshold in rituximab-chemotherapy resistant B-cell lymphoma by targeting mitochondrial proteins

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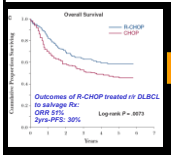
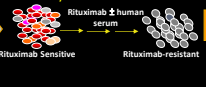
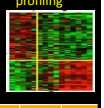
RPCI Lymphoma translational research program



Development of a model to study rituximab resistance



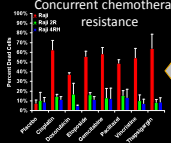
Identification of targets by Gene profiling



Biomarkers of response

Pre-clinical studies of with lead compound

Concurrent chemotherapy resistance

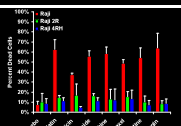


Selected Genes up regulated	2R	4RH
HDAC-1	+2.13	+2.47
Ubiquitin-activating enzyme Ubiquitin-conjugating enzyme	+ 2.32 + 2.33	+ 2.6 + 3.3
Proteasome subunit beta Proteasome 26S	+ 2.17 + 3.18	+ 2.1 + 4.0

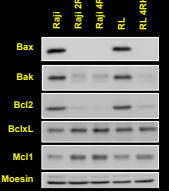
Czuczman et. al. Clin Cancer research 2008;14:1561-70.

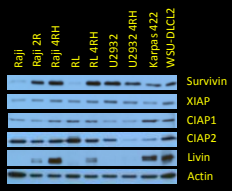
Deregulation of apoptosis and cell cycle

Deregulation of apoptosis in rituximab resistant cell lines

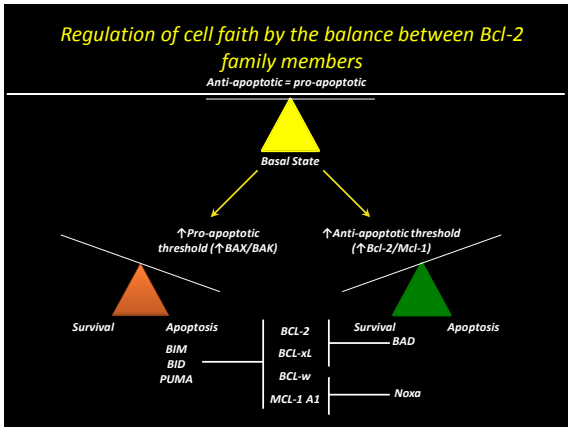


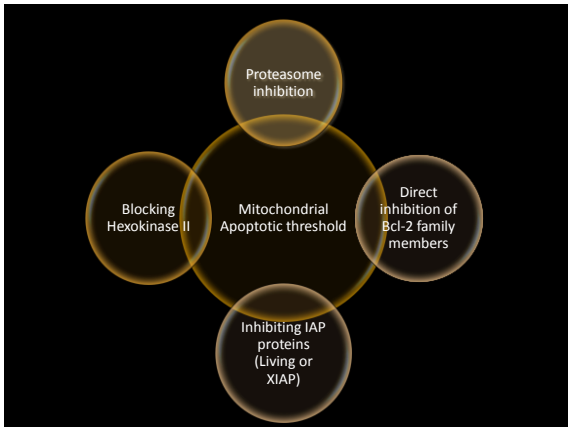
RSCL	RRCL
Raji (Burkitt's)	Raji 4RH
RL (GCB-DLBCL)	RL 4RH
U2932 (ABC-DLBCL)	U2932 4RH



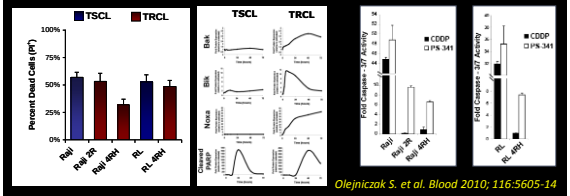


Regulation of cell faith by the balance between Bcl-2 family members



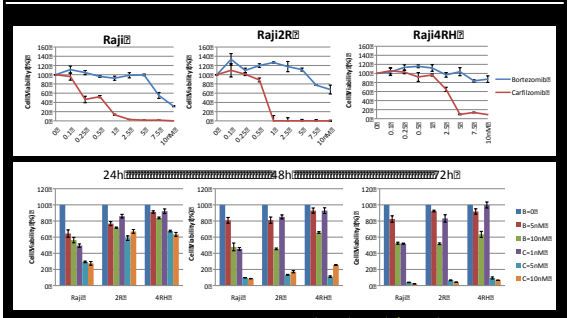


Pharmacological target of the Ubiquitin-Proteaomse system in rituximab-resistant B-cell lymphomas



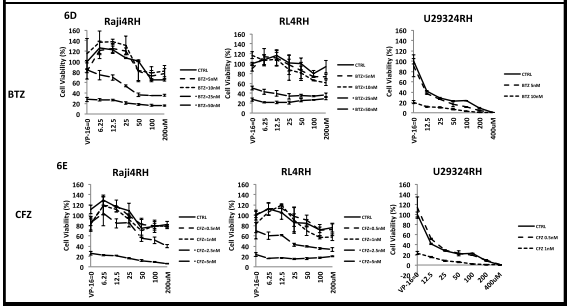
Olejniczak S. et al. Blood 2010; 116:5605-14

Carfilzomib is more potent than bortezomib in RSCL and RRCL



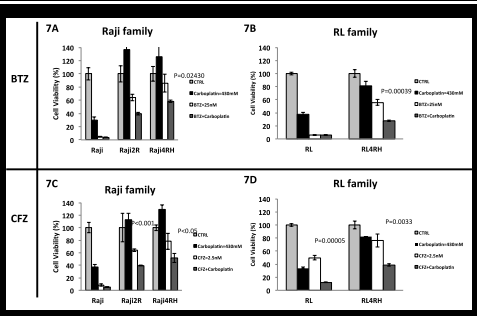
Gu J. et al. British Journal of Hematology; 2013;24:1030-1038

Carfilzomib overcomes rituximab-resistance in combination with VP-16



Gu J. et al. British Journal of Hematology; 2013;24:1030-1038

Carfilzomib potentiates carboplatin anti-tumor activity in both Raji and RL sensitive and resistant cells



Gu J. et al. British Journal of Hematology; 2013;24:1030-1038

Phase I/II Study of Carfilzomib plus Rituximab plus Ifosfamide plus Carboplatin plus Etoposide (C-R-ICE) in Patients with Relapsed/Refractory Diffuse Large B-cell Lymphoma (DLBCL)

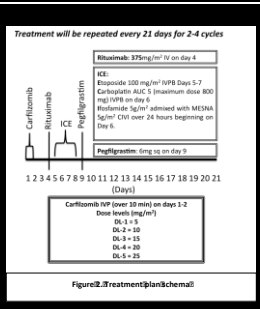
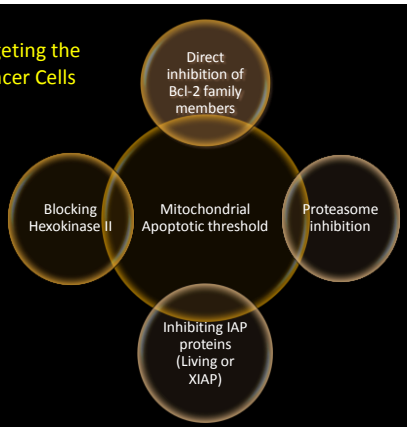
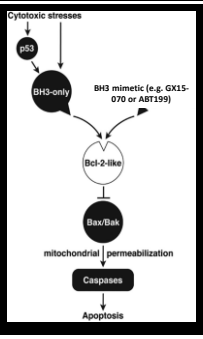


Figure 2. Treatment plan schema

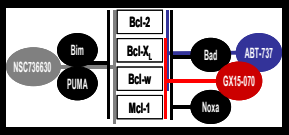
Targeting the Cancer Cells



BH3 mimetic in RRCL

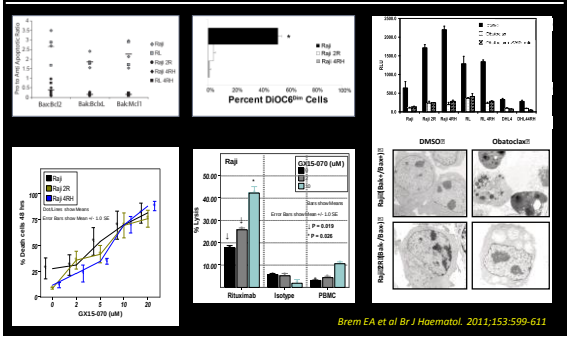


- **Active against multiple B-cell lymphoma cell lines**
- **Enhance the anti-tumor activity of rituximab**
- **Synergize with various chemotherapy agents**



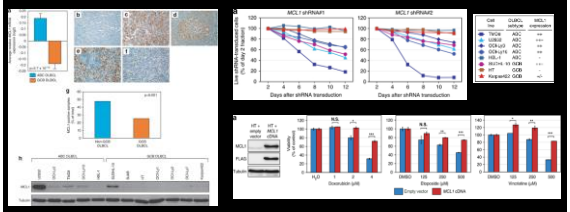
Shoemaker et al Blood 2006; 108:825a
Kitada et al Blood 2006; 108:2487a
Brem EA et al Br J Haematol. 2011;153:599-611

Executing alternative pathways of cell death in rituximab-resistant lymphomas using BH3 mimetics



Brem EA et al Br J Haematol. 2011;153:599-611

MCL1 is deregulated in subgroups of diffuse large B-cell lymphoma and confers resistance to chemotherapy agents



Wenzel et al. Leukemia, 2013; 27:1381-1390

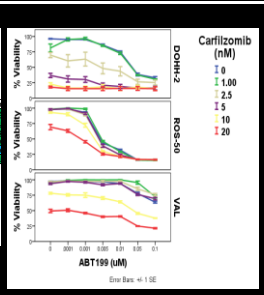
CFZ and ABT-199 combination results in synergistic effects in double hit DLBCL cell lines

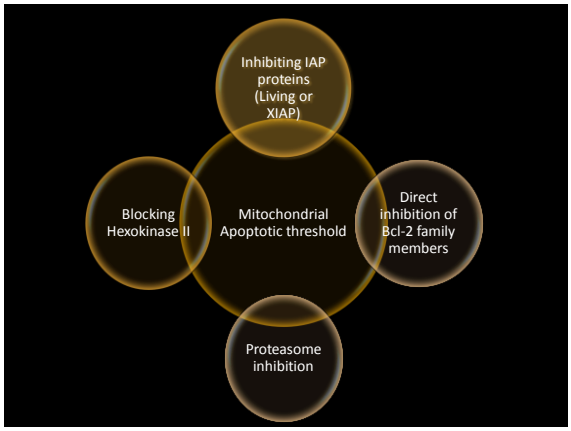
DOHH-2		CFZ<3	2.5	5	10	20
ABT-199	0.0001	3.533	0.712	0.488	0.465	0.878
	0.001	1.538	0.812	0.472	0.464	0.882
	0.005	2.691	0.495	0.308	0.53	0.865
	0.01	1.087	0.429	0.277	0.443	0.904
	0.05	0.23	0.234	0.241	0.522	0.91
	0.1	0.201	0.247	0.235	0.456	0.869

ROS-50		CFZ<3	2.5	5	10	20
ABT-199	0.0001	771.5	274.424	75.949	0.908	0.63
	0.001	729.04	1.139	1.517	0.515	0.501
	0.005	0.087	0.095	0.144	0.159	0.338
	0.01	0.05	0.055	0.098	0.163	0.306
	0.05	0.048	0.074	0.106	0.137	0.295
	0.1	0.081	0.115	0.146	0.203	0.351

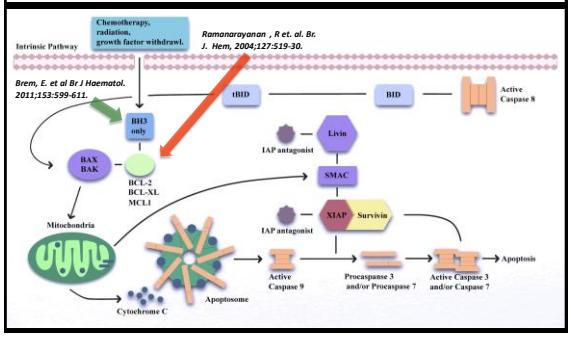
VALB		CFZ<3	2.5	5	10	20
ABT-199	0.000					
	1	10.141	23.559	6.084	0.593	0.456
	0.001	80.718	5.169	2.992	0.584	0.388
	0.005	67.725	1.174	1.034	0.486	0.317
	0.01	126.486	6.248	1.694	0.358	0.325
	0.05	4.428	0.744	0.53	0.224	0.181
	0.1	0.388	0.578	0.497	0.185	0.159

CI >10	Very strong antagonism
CI >1	Antagonistic
CI .7-.99	Slight/Moderate Synergism
CI .3-.7	Synergism
CI <.3	Strong Synergism





The role of anti-apoptotic proteins



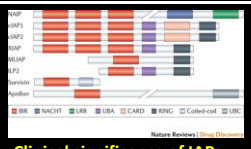
The regulation of caspase activation by the balance between IAPs and SMAC

IAPs

- 8 currently identified IAP proteins
- Characterized by Baculoviral IAP repeat (BIR) domain
- BIR domains can bind and sequester Caspases
- XIAP, cIAP1, cIAP2, and livin also contain an E3 unbiquitin ligase RING domain

SMAC

- SMAC released from mitochondria after membrane permeabilization
- Interacts with IAP proteins through BIR domains by means of Ala-Val-Pro-Ile sequence
- Promotes release of caspases 3, 7, and 9

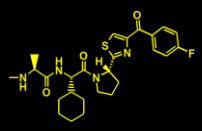


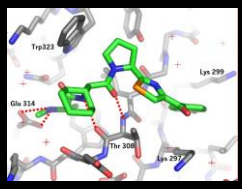
Clinical significance of IAPs expression in lymphomas

- XIAP is overexpressed in >50% DLBCL and correlates with poor prognosis
- cIAP2 overexpression from the t(11;18) translocation is common in MALT lymphoma
- Reed-Sternberg cells show high XIAP expression
- High survivin expression correlates with poor outcomes in lymphoma and leukemia pts

Fulda S et al. Leukemia, 2009; 23:467-476

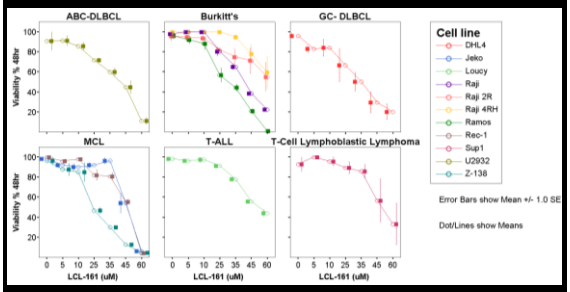
LCL-161



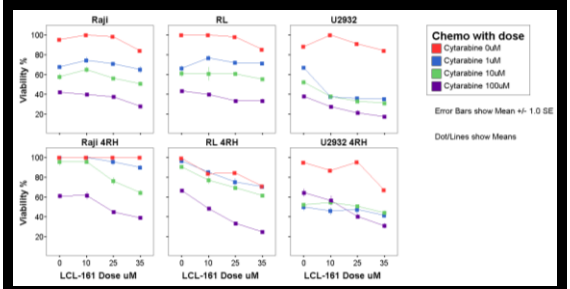


- Small molecule mimetic of SMAC developed by Novartis
- Orally bioavailable pan-IAP inhibitor
  - XIAP IC_{50} : 13nM
  - cIAP IC_{50} : 2nM
- Antagonizes the IAP caspase interaction releasing active caspases and promoting apoptosis

In vitro exposure of NHL and Leukemia cell lines to LCL-161 results in dose dependant anti-tumor activity

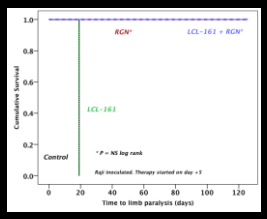


LCL-161 enhances the anti-tumor activity of cytarabine in rituximab-chemotherapy resistant cells

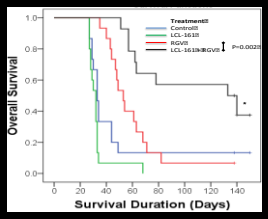


LCL-161 enhances the activity of rituximab/gemcitabine and vinoralbine (RGN) in rituximab-resistant lymphoma *in vivo*

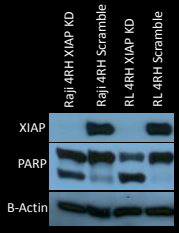
Rituximab-chemotherapy sensitive lymphoma in vivo mode

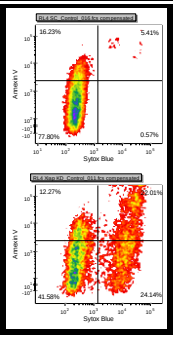


Rituximab-chemotherapy resistant lymphoma in vivo mode

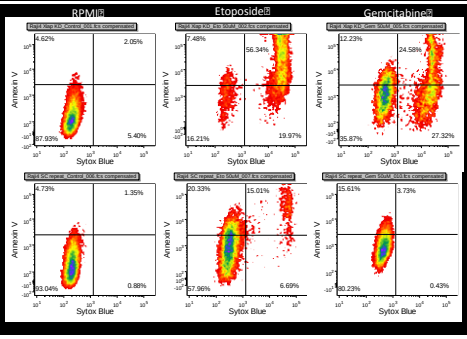


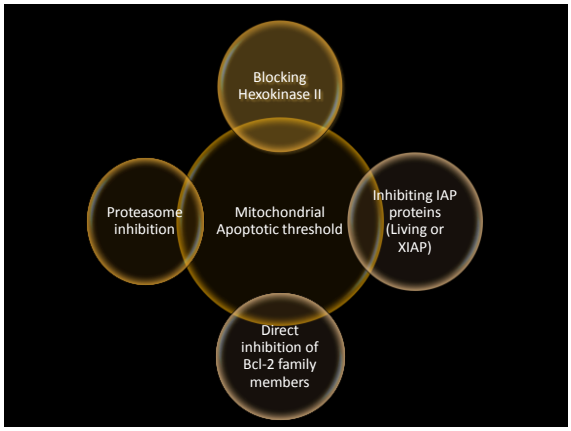
Effects of XIAP knockdown in RRCLs



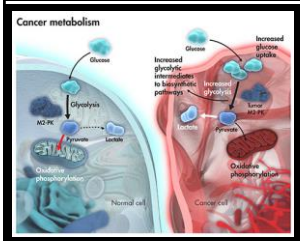


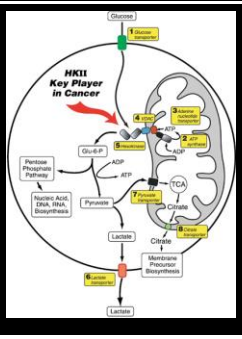
Effects of XIAP knockdown in rituximab-chemotherapy resistant cells





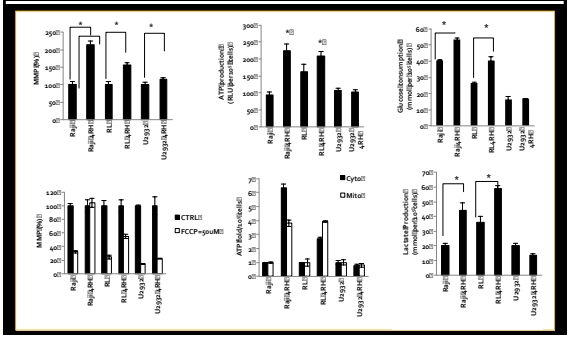
Deregulation of glucose metabolism in cancer



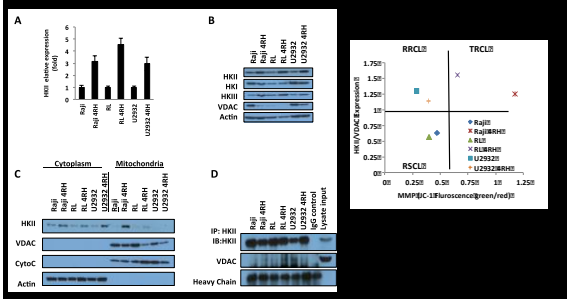


Pelicano et al. Oncogene 2006 25:4633-4646

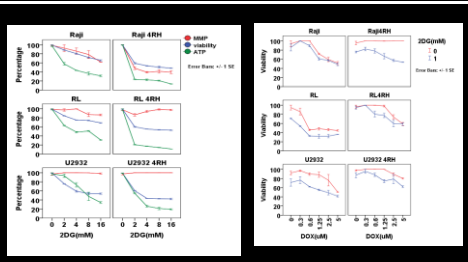
Enhanced mitochondria membrane potential and glucose metabolism in RRCL



HK2 mRNA and protein levels in RSCL and RRCL



Distinct effects of hexokinase inhibition in therapy-resistant (TRCL), rituximab-resistant (RRCL) and rituximab-sensitive cell lines (RSCL)



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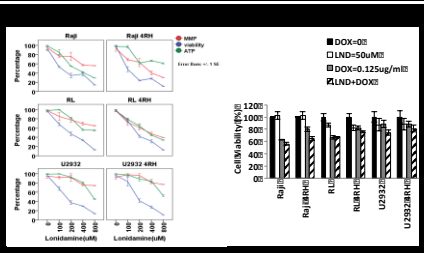
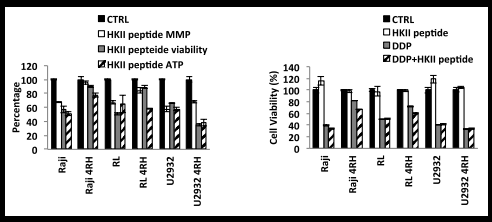
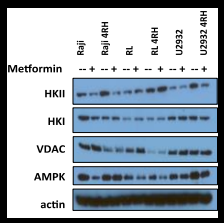
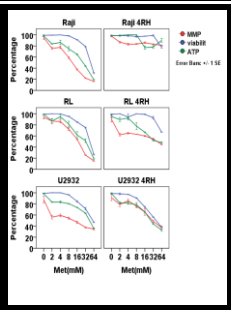


Distinct effects of hexokinase inhibition in therapy-resistant (TRCL), rituximab-resistant (RRCL) and rituximab-sensitive cell lines (RSCL)

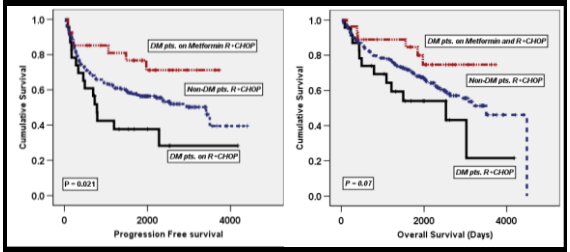


Effect of blockage HKII glycolytic function by 2DG in RSCL and RRCL

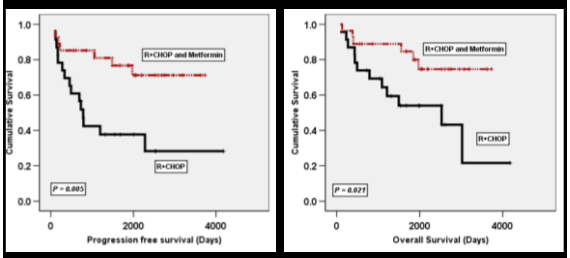




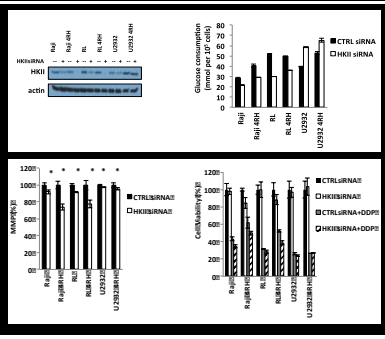
Positive impact of metformin in the clinical outcomes of DLBCL patients treated with R+CHOP at RPCI (N=275pts)



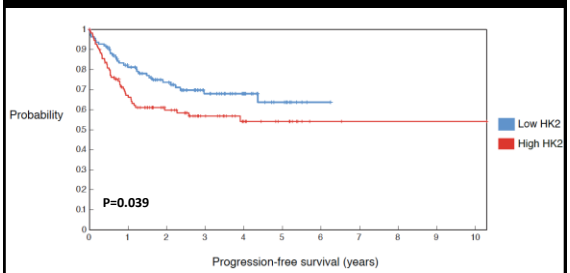
Positive impact of metformin in the clinical outcomes of DM DLBCL patients treated with R+CHOP at RPCI (N=50pts)



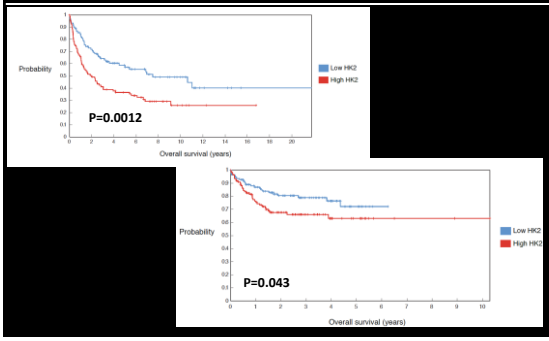
HKII is required for drug resistant phenotype



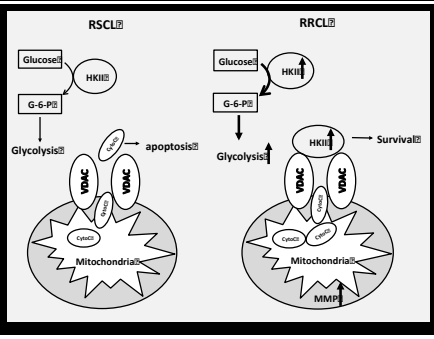
Effect of HKII expression in the PFS of newly diagnosed DLBCL treated of R+CHOP (N= 220)

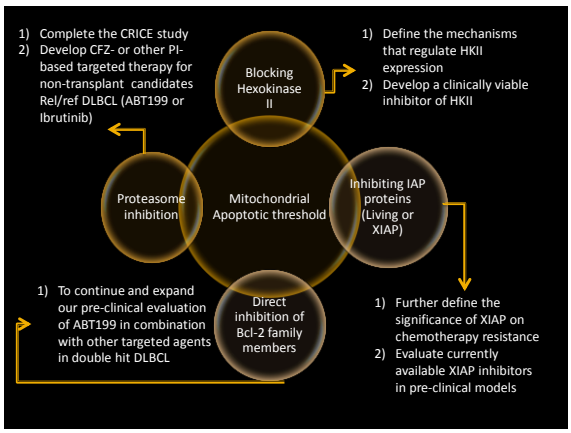


Effect of HKII expression in the OS of newly diagnosed DLBCL treated with CHOP (N=181) of R+CHOP (N=220)



Proposed mechanism of resistance and altered metabolism in RRCL





In summary

- Ongoing studies defining distinct regulatory pathways between GCB and ABC DLBCL will aid in the incorporation of novel small molecule inhibitors in the treatment of DLBCL
  - Lenalidomide, Bortezomib and Ibrutinib appear to be more active in ABC DLBCL subtypes
  - Ongoing drug screening programs are seeking to identify novel agents in GCB- and double hit-DLBCL
- A sub group of DLBCL patients over-expressing Bcl-2 and c-Myc with poor clinical outcome had been identified. Such patients need to be treated with more aggressive regimens, CNS prophylaxis, followed by HDC-ASCS