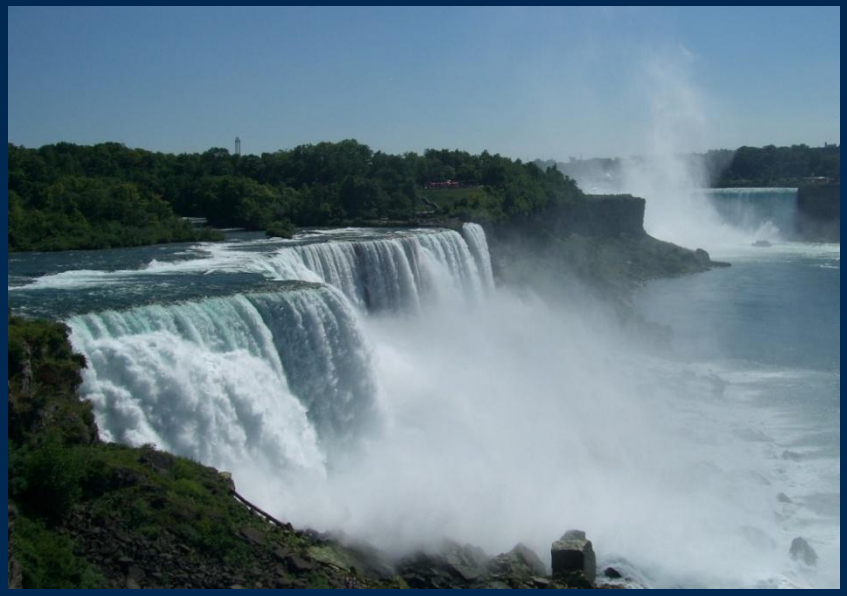
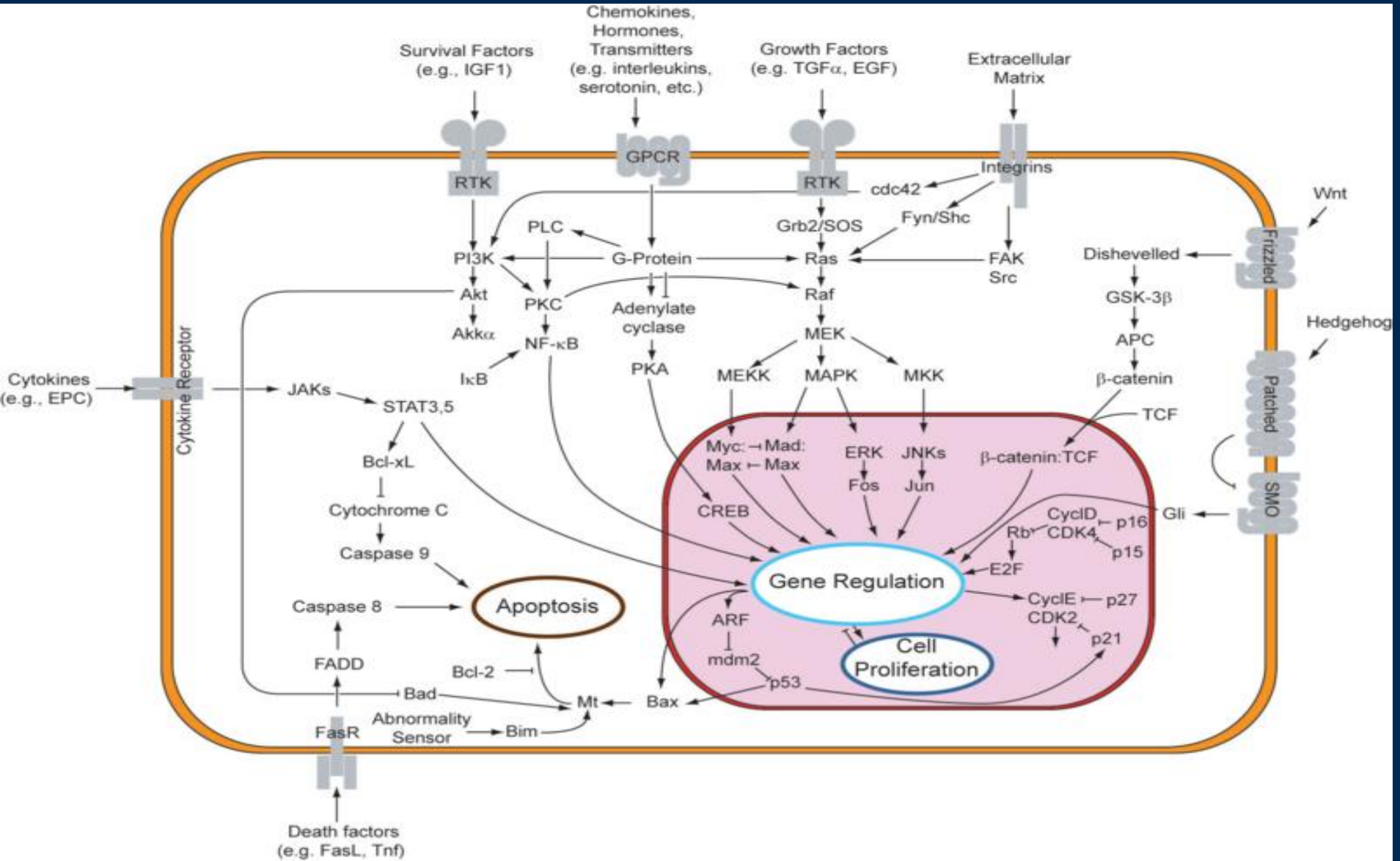


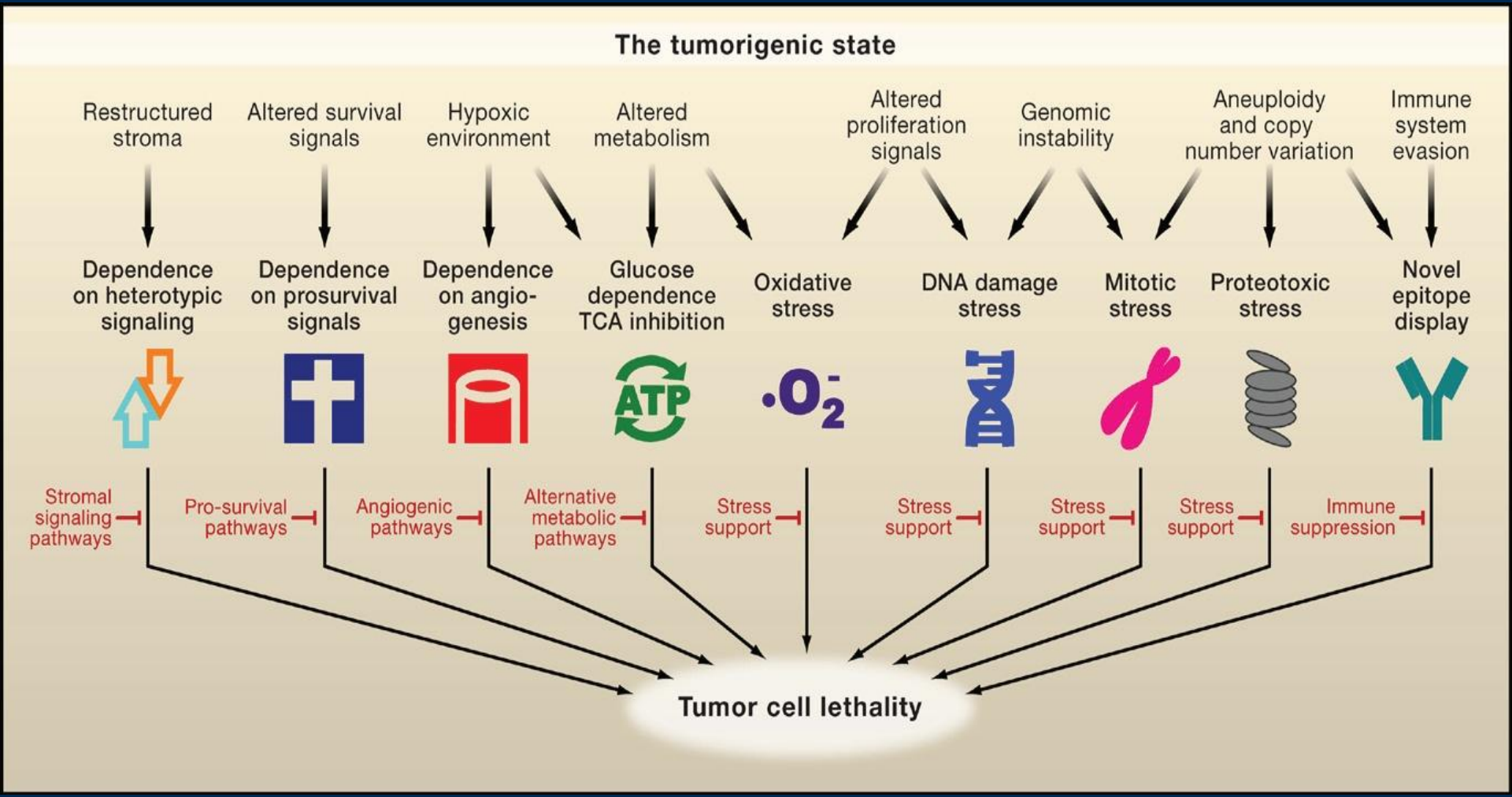
SMAC Mimetics as new Anticancer Agents

Alex A. Adjei,
Roswell Park Cancer Institute
Buffalo, NY
USA

ROSWELL PARK CANCER INSTITUTE

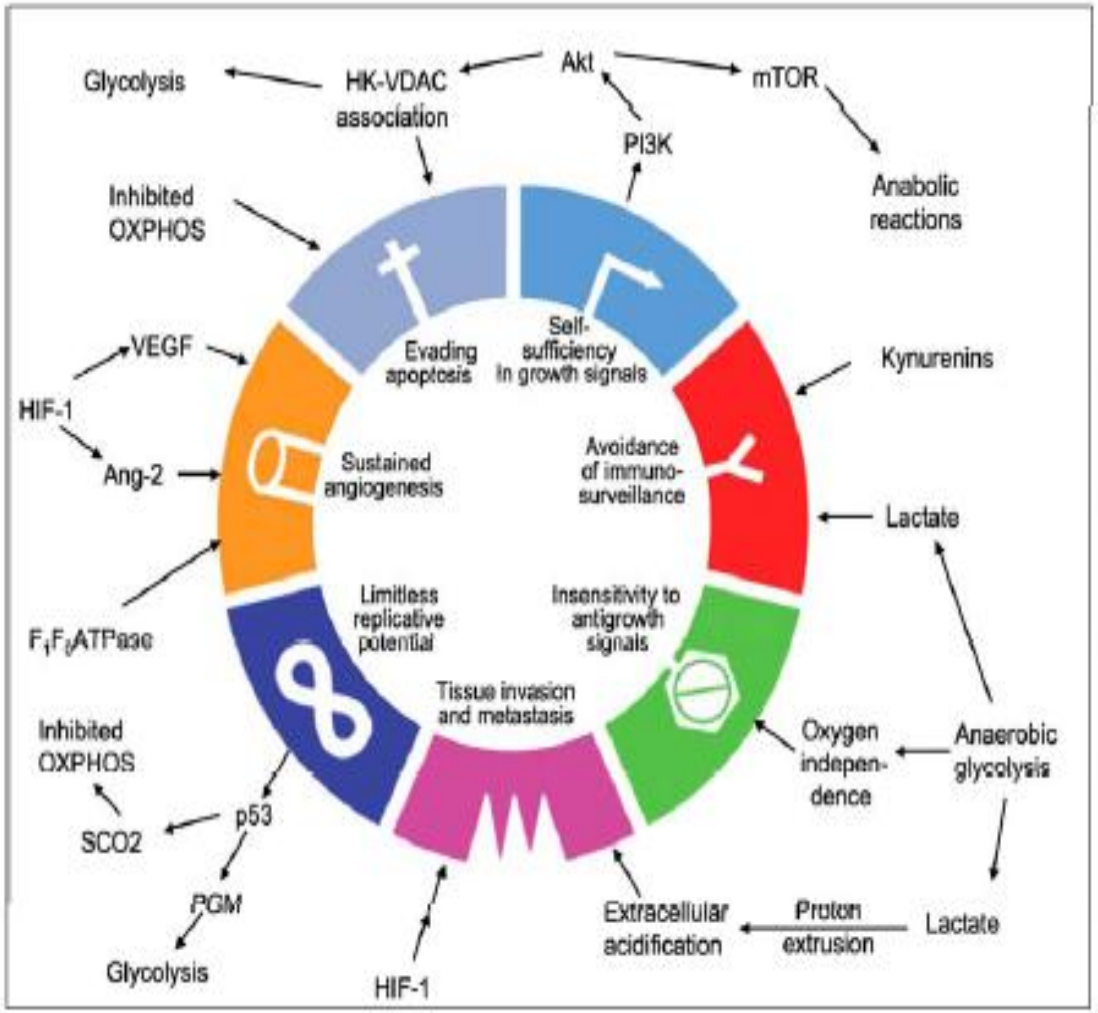
Overview of Signal Transduction Pathways





Luo et al, Cell 2009

Centrality of mitochondria to cancer cell function and survival

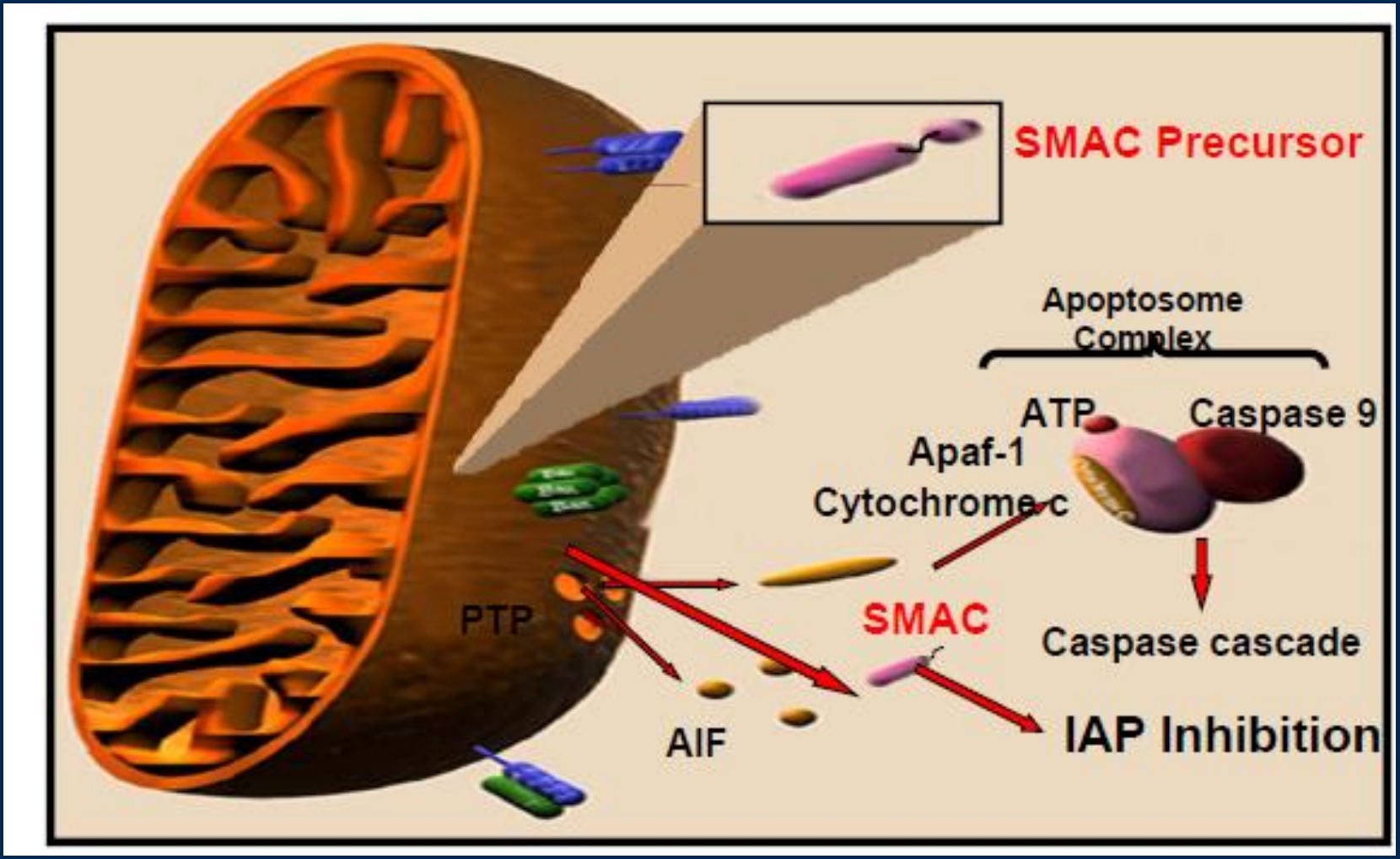


Kroemer and Pouyssegur, Cancer Cell 2008

Inhibitors of Apoptotic Proteins (IAPs)

- IAPs inhibit the apoptotic cascade by
  - Blocking caspase activation
  - Promoting proteosomal degradation of caspases
- 8 IAPs (XIAP, cIAP-1, cIAP-2, IAP-2, ML-IAP, NAIP, Survivin, Apollon) described
- IAP expression correlated to chemoresistance and tumor aggressiveness
- Rational Targets for Cancer Therapy

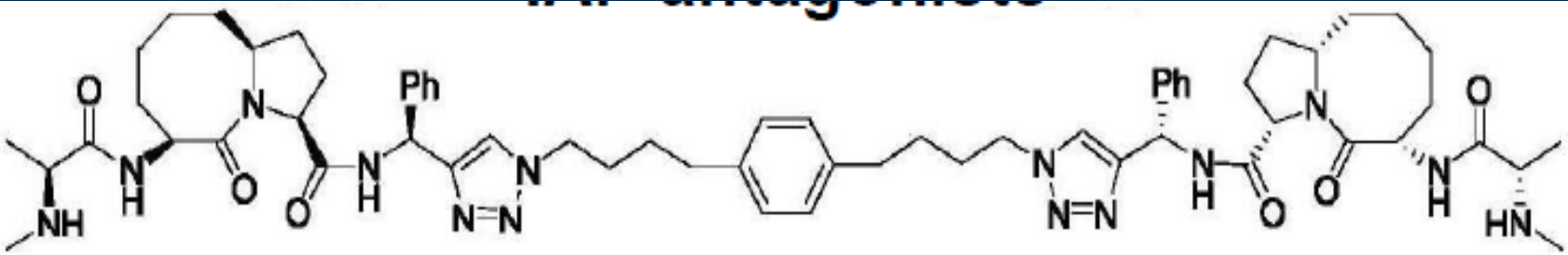
SMAC – Second Mitochondrial-derived Activator of Caspases



SMAC Overview

- **Smac (second mitochondrial-derived activator of caspases)**
  - Released from mitochondria upon receipt of cell death stimulus
  - interacts with multiple Inhibitor of Apoptosis Proteins (IAPs):
    - Relieves their inhibitory effect on caspases
    - Inhibits NF-kB activation affecting tumor growth, survival and metastases
- **Smac mimetic drugs are conceptually unique**
  - Relevant to treating all types of cancer by targeting fundamental mechanisms of cancer cell survival and resistance
  - Target survival and resistance proteins that are downstream from other cancer therapy targets enabling synergy with many other therapies

SMAC Mimetics are IAP Antagonists



SM-164

(IC_{50} < 1nM)

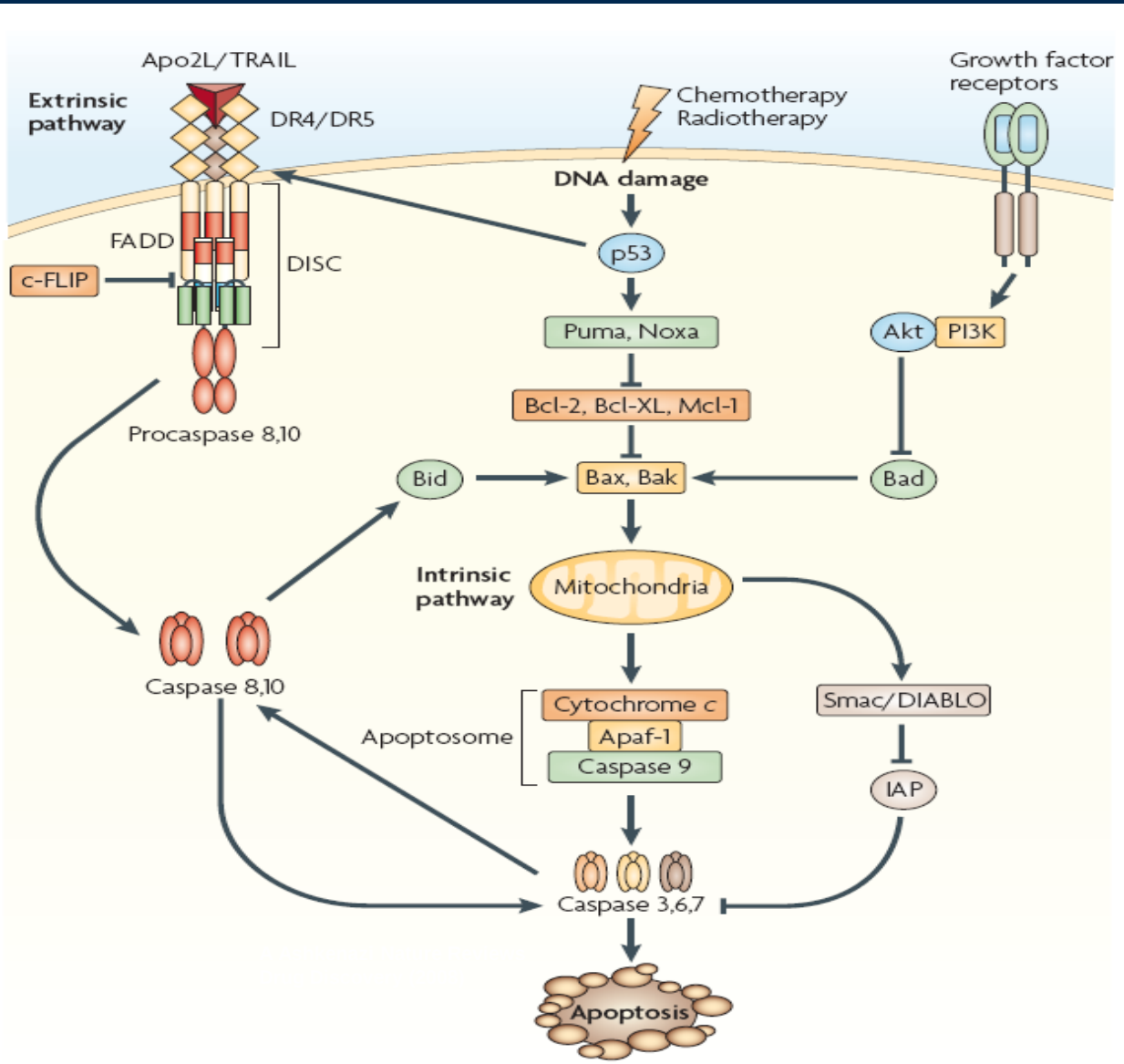
c-IAP1 c-IAP2 XIAP

Inhibitors of Apoptosis

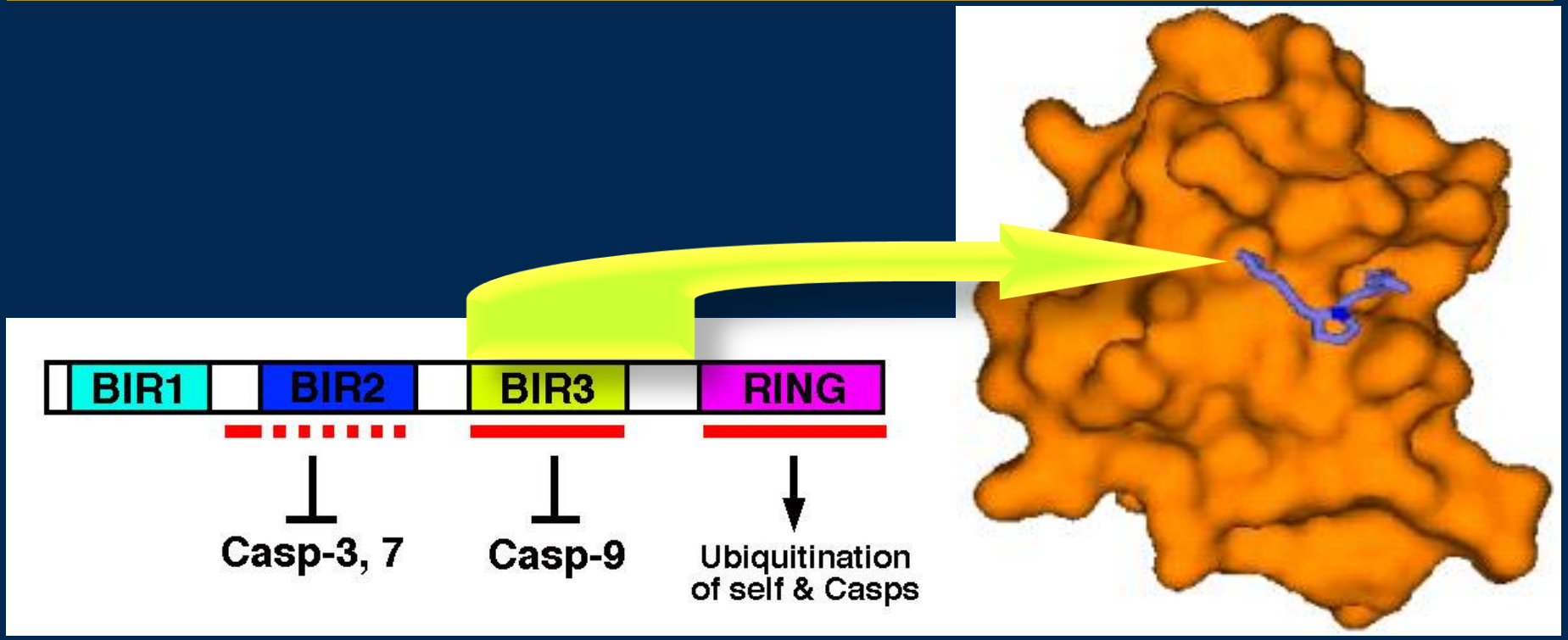
SMAC Mimetics

Activating Apoptosis Downstream of Most Cancer Therapies

- **Rationally-designed therapeutics that target fundamental anti-apoptotic mechanisms of tumor cell survival and resistance to apoptosis**
- **Apoptosis is the main mechanism through which innate responses and therapies destroy cancer cells.**
- **SMAC mimetics act alone or with other therapies to overcome pivotal cancer resistance mechanisms.**



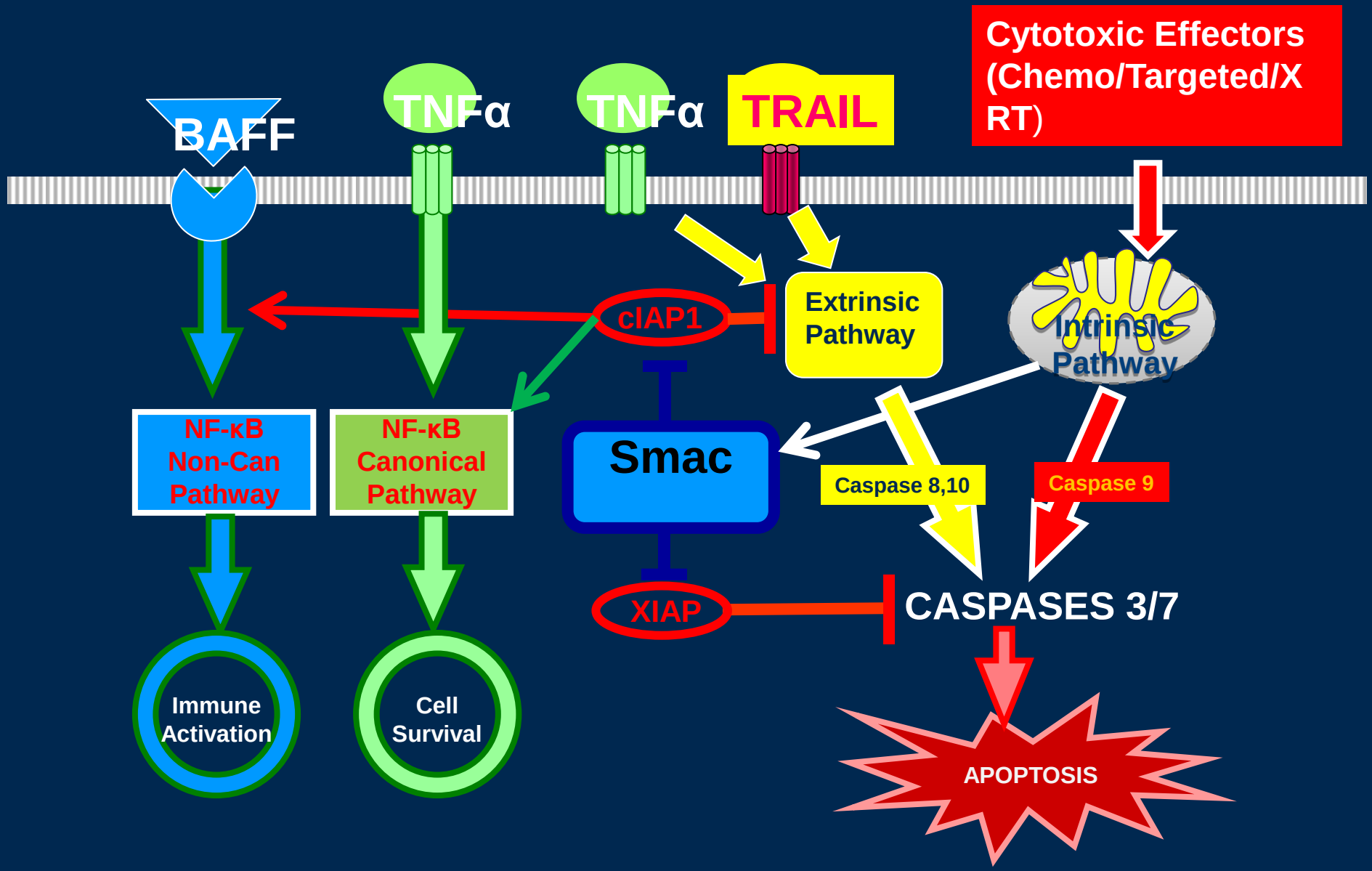
All Smac Mimetics are Based on the Structure of the N-terminal Tetrapeptide of Native Smac



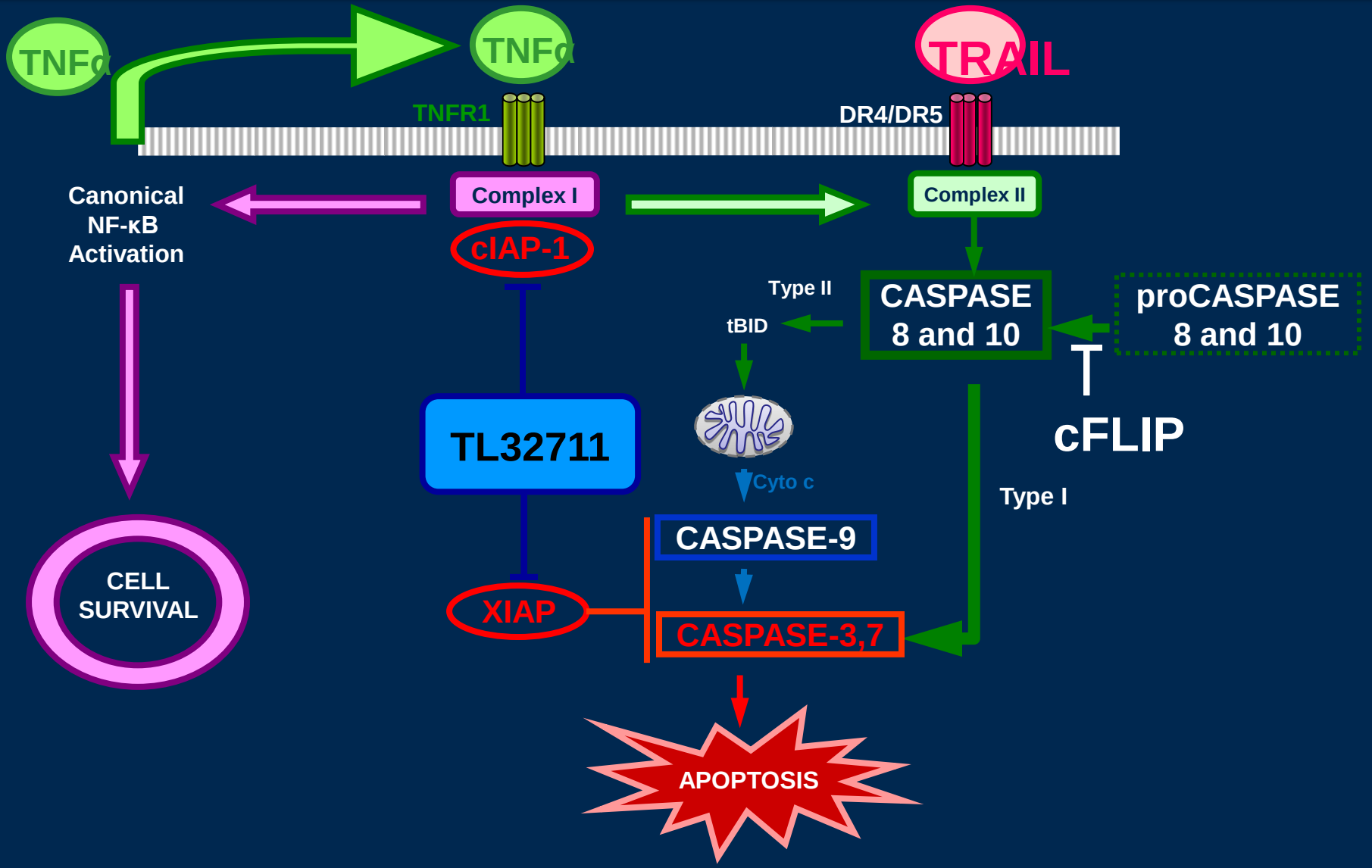
SMAC – Second Mitochondria-derived Activator of Caspases

SMAC AVPI-tetrapeptide binds to surface groove on XIAP BIR3 domain

Smac - Mechanism of Action



SMAC Mimetics Mimic the Activity of Smac – the Endogenous IAP Inhibitor



Effect of SMAC Mimetics on NFκB Pathways

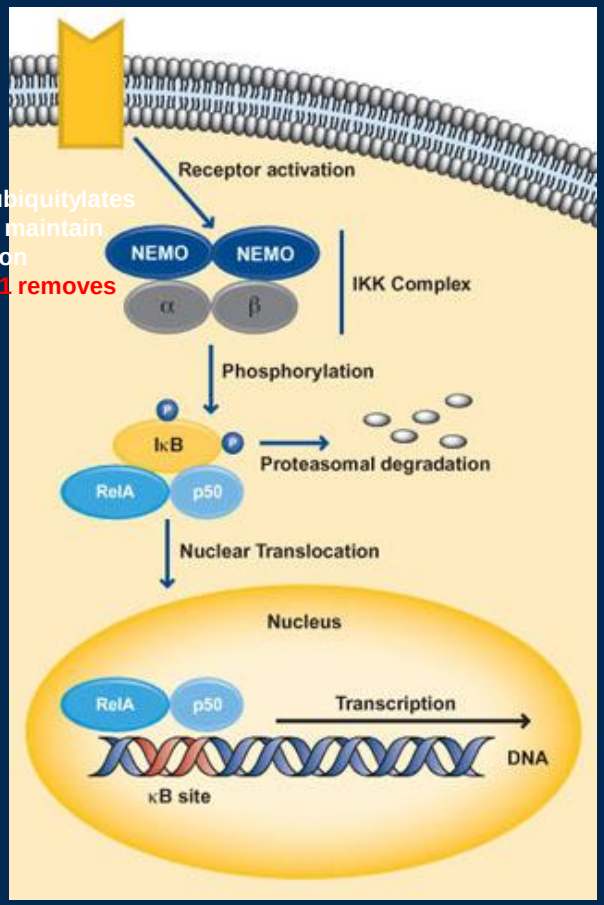
Canonical Pathway

Rapid inhibition of canonical pathway

Non- canonical Pathway

cIAP1 ubiquitylates RIP1 to maintain activation

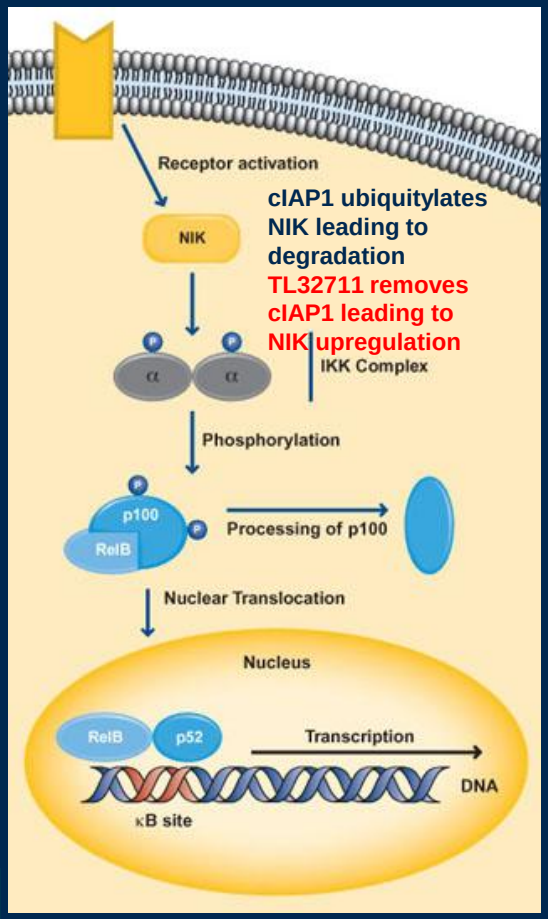
TL32711 removes cIAP1



Inflammation

SMAC Mimetic

Slow activation of non-canonical pathway



Lymphoid Development

SMAC Mimetics in Clinical Trials

Company	SMC	Condition
Aegera / Human Genome Sciences	AEG40826/ HGS1029	Advanced solid tumours / relapsed or refractory lymphoid malignancies (iv)
Ascenta	AT-406	Advanced solid tumours and lymphomas (po)
Genentech / Roche	GDC-0152/ RG7419	Locally advanced or metastatic malignancies (iv)
Novartis	LCL161	Solid tumours (po, weekly and daily)
Tetralogic	TL32711	Advanced or metastatic solid tumours / refractory solid tumours or lymphoma (iv)

TL32711 Overview

- Potent IAP selective dimer:
  - Preferential suppression of cIAP1, but also inhibits cIAP2 , XIAP, and ML-IAP but no effect on Survivin
  - Marked synergy with TNFα and TRAIL in vitro
  - Broad combination synergy with chemotherapy without increased toxicity
  - Favorable ADME characteristics
  - Good pharmaceutical properties for IV dosing

TL32711 Mechanism of Action

- Selectively binds to cIAP1 *vs.* cIAP2 and induces rapid autodegradation of cIAP1 in all cell types
  - Following treatment of **sensitive** tumors
    - Autocrine TNF and exogenous TNF and TRAIL bind to death receptors
    - Cytokines activate pro-apoptotic pathway via caspase-8 activation
    - Elevated procaspase-8 levels in tumors provide tumor selectivity for killing
    - Rapidly shuts off canonical NFkB pro-survival pathway
    - cIAP2 degradation via cIAP1 ubiquitinylation not required for killing
  - Following treatment of **normal cells**
    - cIAP2 can replace cIAP1 function and may play protective role
      - In sensitive tumor cells, strong apoptotic signaling overwhelms any cIAP2 effect
- Binds to and antagonizes XIAP function in all cells
  - De-represses caspase-9 inhibition activity generated via intrinsic pathway
  - De-represses executioner caspase-3 and -7 activity that represents the final block in apoptosis pathway

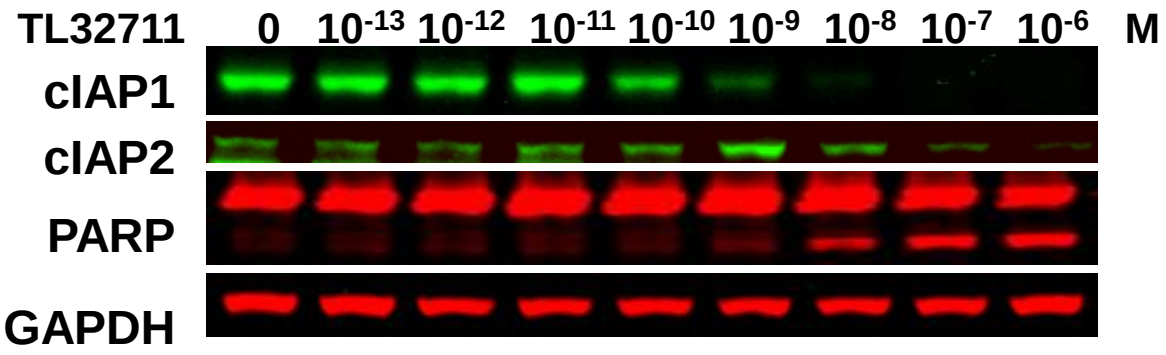
TL32711 Key Preclinical Efficacy Data

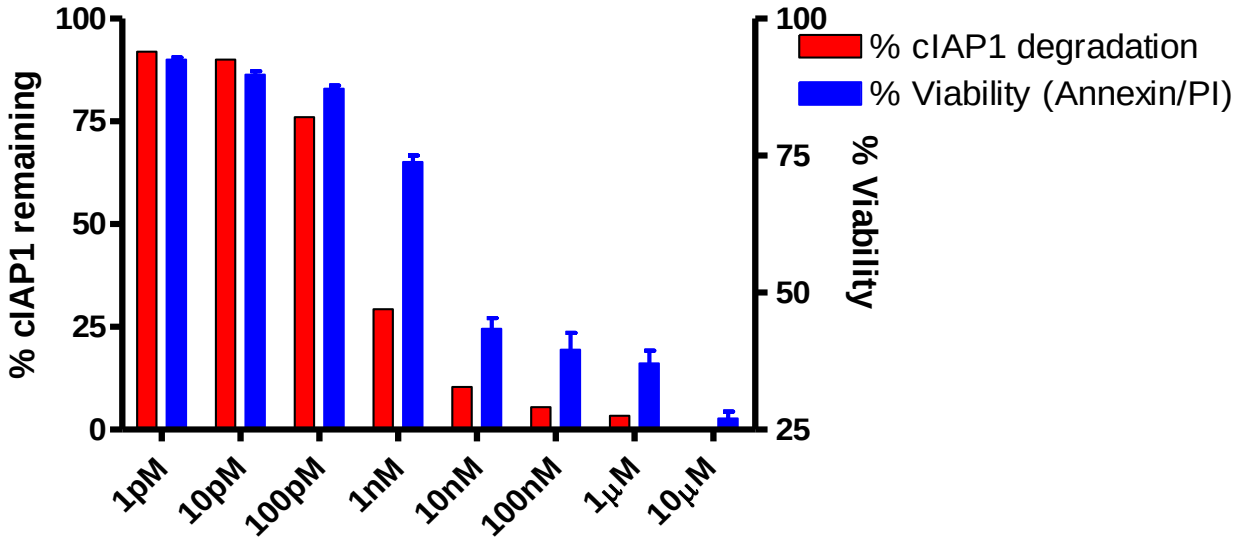
- Rapid and potent target suppression of IAPs
  - Occurs in all cell types and tissues within 1 hr of drug exposure
  - Prolonged target suppression in tumor vs. normal tissues
  - PK/PD relationship between target suppression and anti-tumor activity
- TL32711 activity in multiple tumor models
  - Activity in both primary tumor and established tumors
  - Activity in hematologic and solid tumor models
  - Complete regressions with prolonged survival in xenograft models
  - *In vivo* efficacy retained in larger tumors
- Potent synergy with TNFα and TRAIL
- Potent synergy with multiple chemotherapies

cIAP1 Suppression Correlates to Cancer Cell Death

MDA-MB-231 Breast Tumor Cells

24 hr Treatment

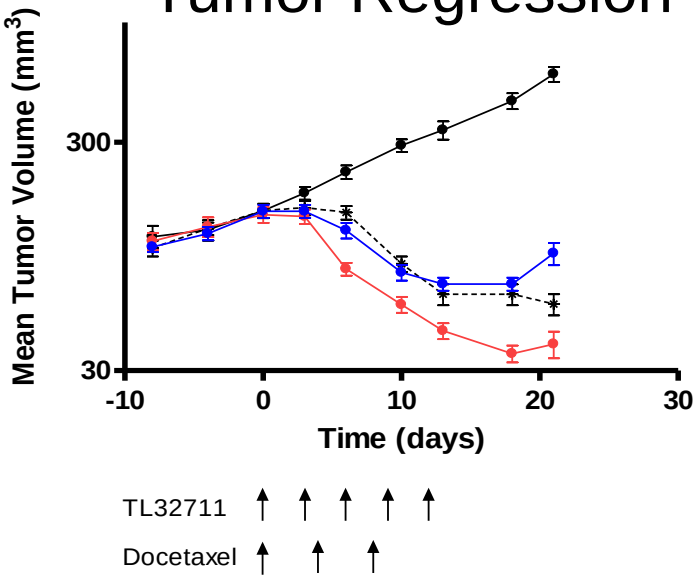




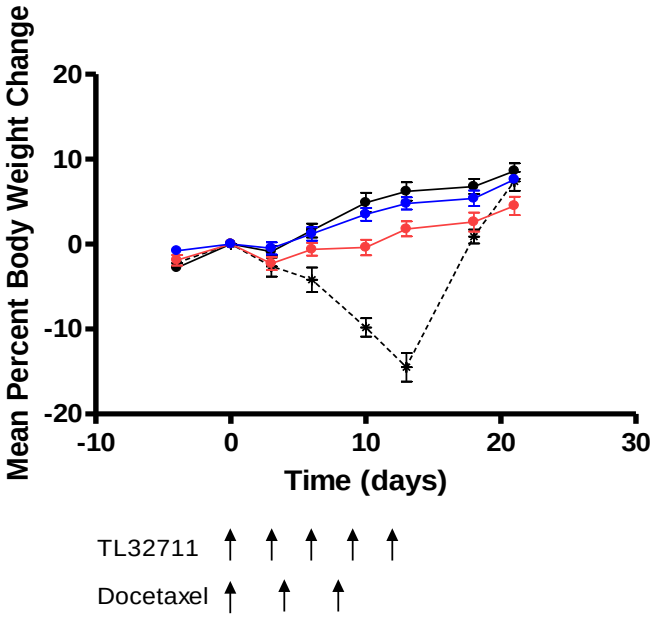
TL32711 Causes Tumor Regressions at Well Tolerated Doses in a MDA-MB-231 Xenograft Model

Efficacy Greater than Docetaxel Administered at the MTD

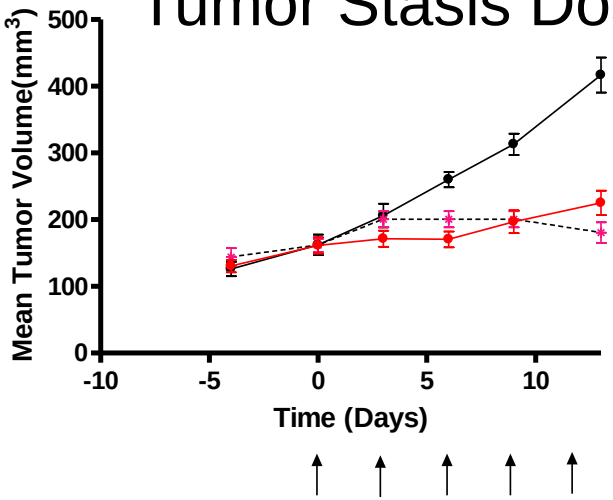
Tumor Regression Dose



- Vehicle Control (12.5% captisol; IP; Q3Dx5)
- Docetaxel (20 mg/kg; IV; Q4Dx3
- TL32711 (10 mg/kg; IP; Q3Dx5)
- TL32711 (5 mg/kg; IV; Q3Dx5)

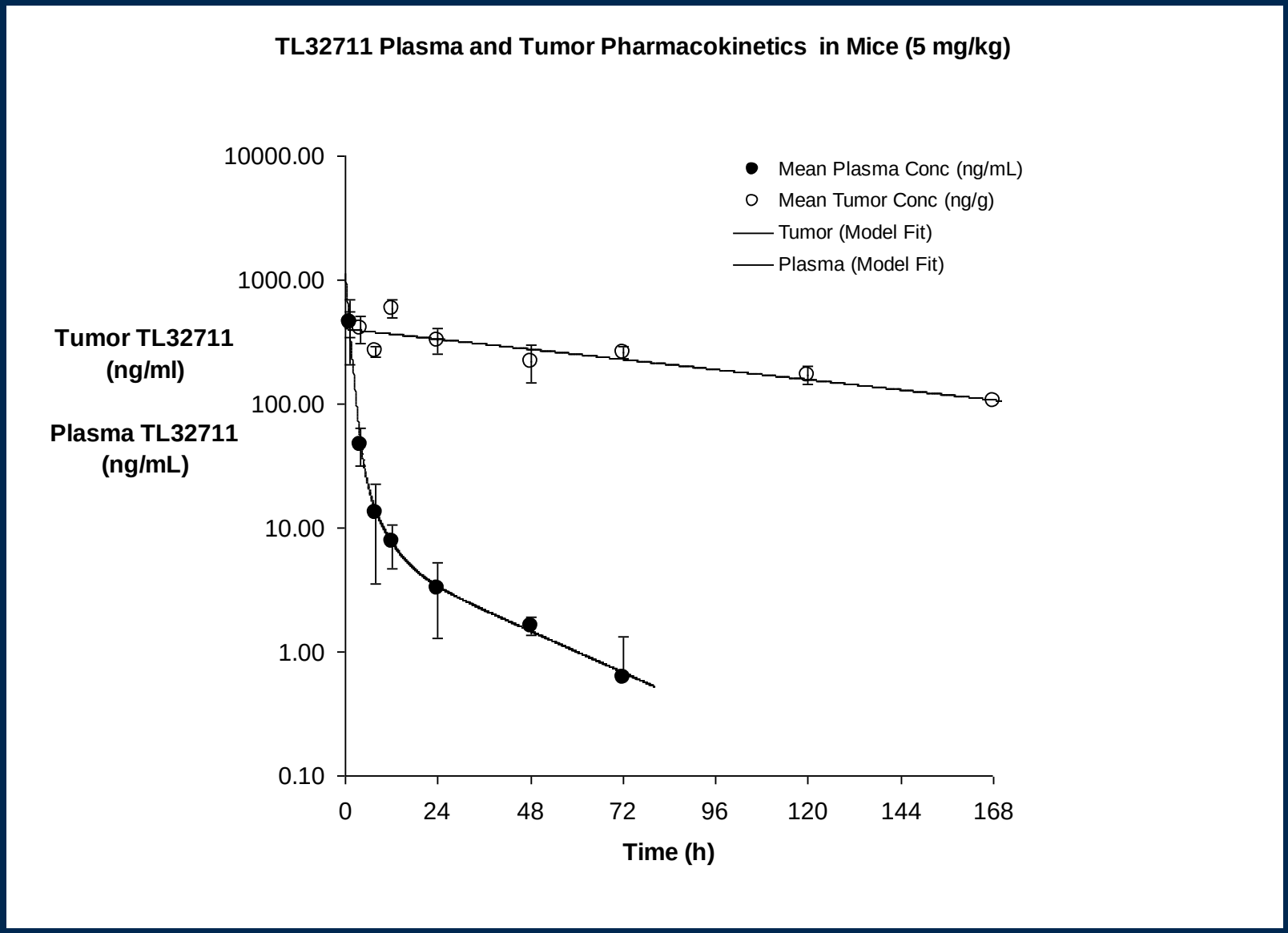


Tumor Stasis Dose

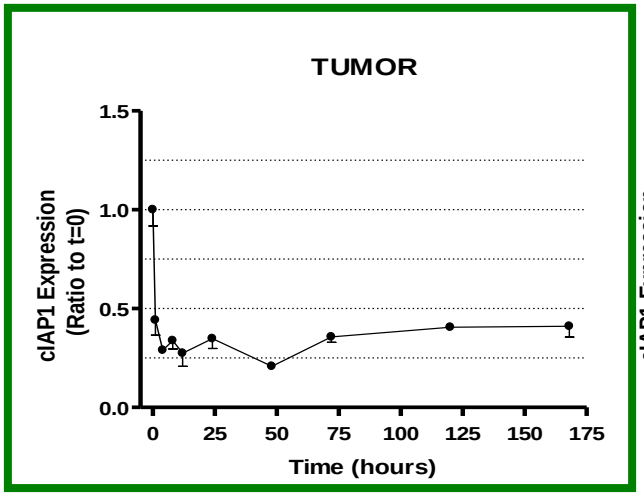


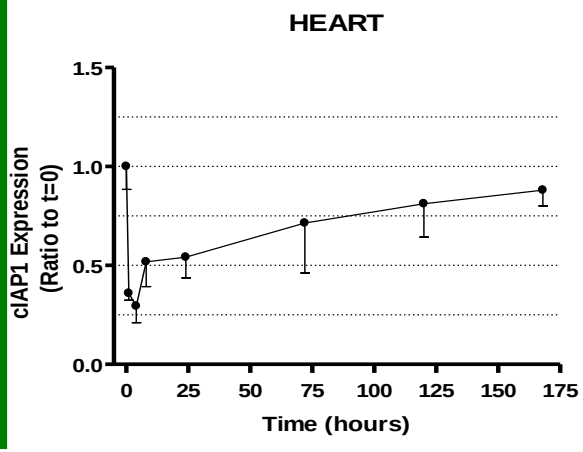
- Vehicle
- Docetaxel 20 mg/kg (q4dx3x2)
- TL32711 1.25 mg/kg

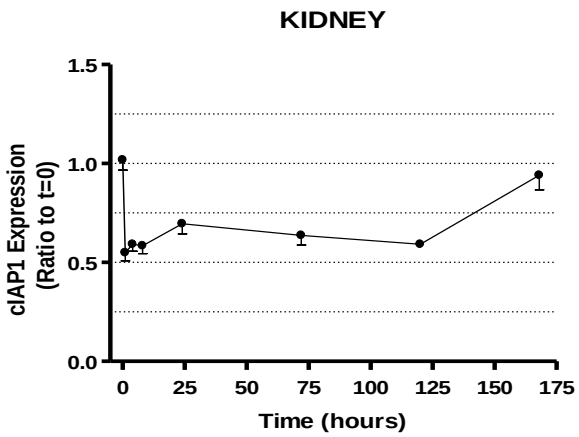
TL32711 Plasma and Tumor Pharmacokinetics in Mice

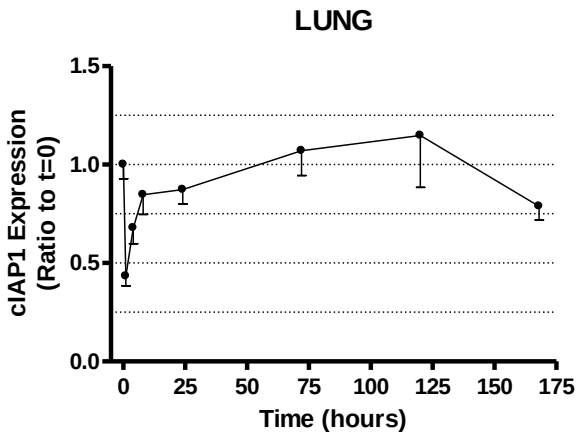


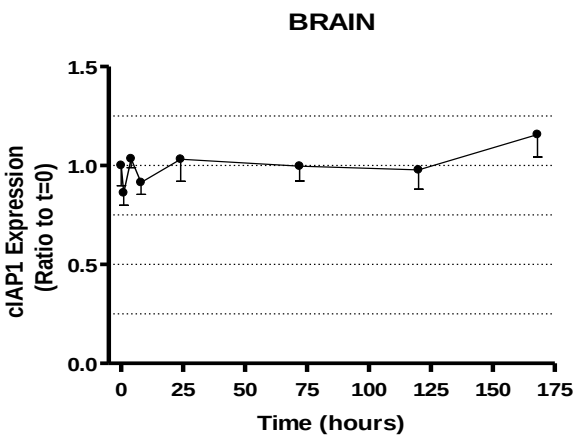
TL32711-induced cIAP1 Reduction is Prolonged in Tumor *vs.* Normal Tissue

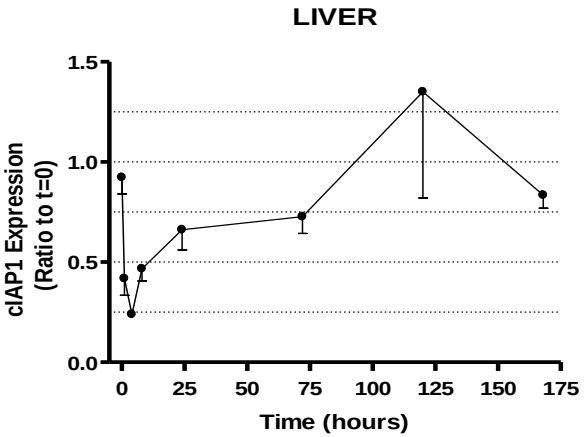












MDA-MB-231 xenograft following single 5 mg/kg dose

TL32711 Phase I Trial

ROSWELL PARK CANCER INSTITUTE

FOX CHASE CANCER CENTER

UNIVERSITY OF PENNSYLVANNIA CANCER CENTER

Measures of Target Coverage & Clinical Effect

- PBMC assay of cIAP-1 as surrogate of tumor cIAP-1 suppression
- Correlation of target suppression with Phase 1 study data
  - Apoptosis activation - circulating cleaved cytokeratin-18 levels and activated caspase-3 levels in serum
  - Clinical measures
    - Radiographic assessment - CT/MRI/FDG-PET
    - Surrogate clinical markers *e.g.* CEA, CA-125
- Correlation of Phase 1 PBMC target suppression drug concentration levels with non-clinical drug concentrations related to target suppression and efficacy

Degradation of cIAPs by TL32711 in Volunteer PBMCs

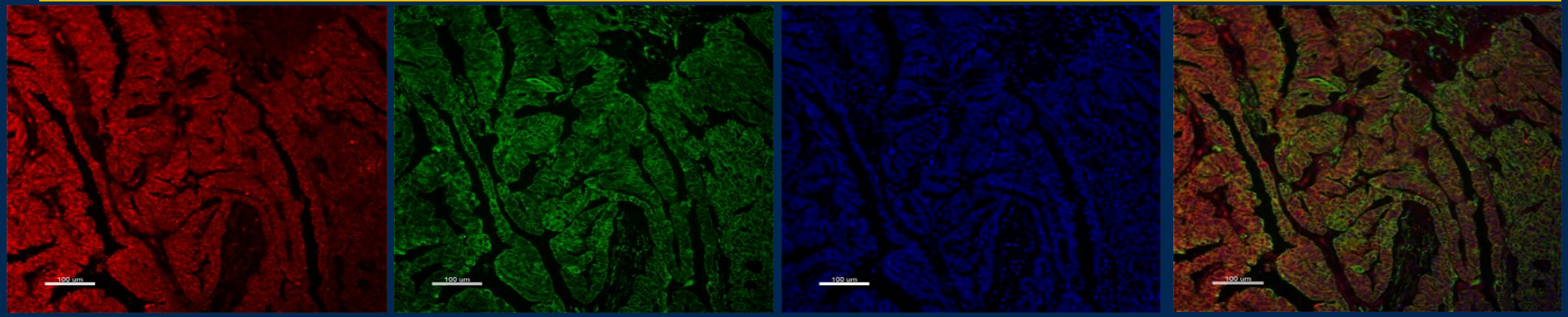
AQUA IHC For Predictive Biomarkers

- Study of 11 different tumor types representing 1164 patient samples
  - Quantitating TNFα & TRAIL levels in tumor and stroma – data by June
  - Identify tumor types with highest expression levels – candidates for Phase 2 study
  - Profile tumor types of interest for other markers of sensitivity including TNFα and TRAIL receptors, cIAP-1, cFLIP, Bcl family members and pro-caspase-8
- Tumor types included
  - Ovarian – 184 patients
  - Melanoma – 80 patients
  - Breast – 261 patients (Her2+/-, ER+/-, Node +/-)
  - Colorectal – 67 patients
  - Pancreatic -39 patients
  - NSCLC – 156 patients
  - Head & Neck – 181 patients
  - Esophageal – 91 patients
  - Bladder – 32 patients
  - Prostate – 24 patients
  - Kidney – 49 patients

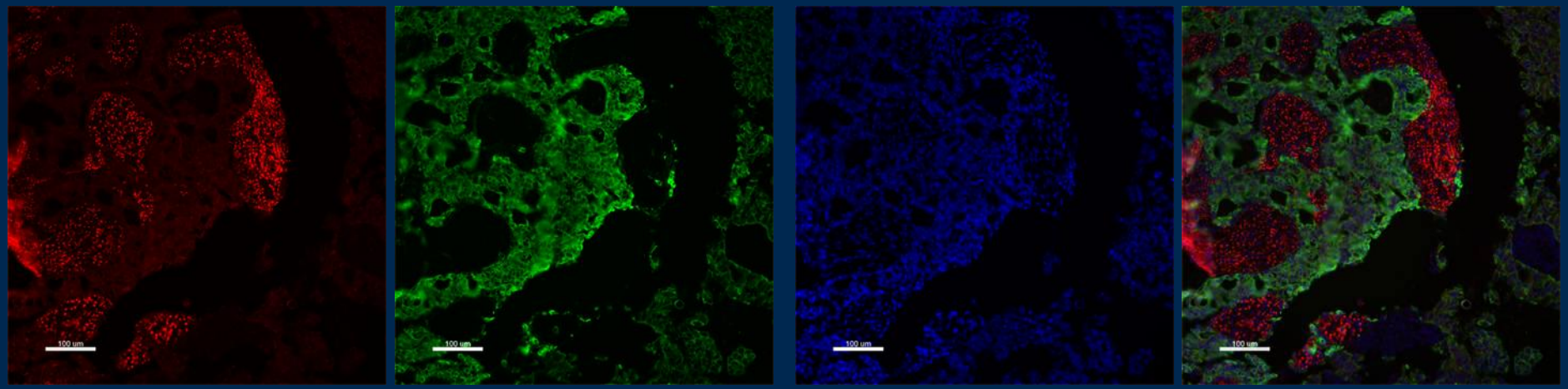
Camp RL, Chung GG, Rimm DL. Automated subcellular localization and quantification of protein expression in tissue microarrays. Nat Med 2002;8:1323 – 7

TNFα Staining in Ovarian Tumor

TNFα | Cytokeratin | Dapi | Combination



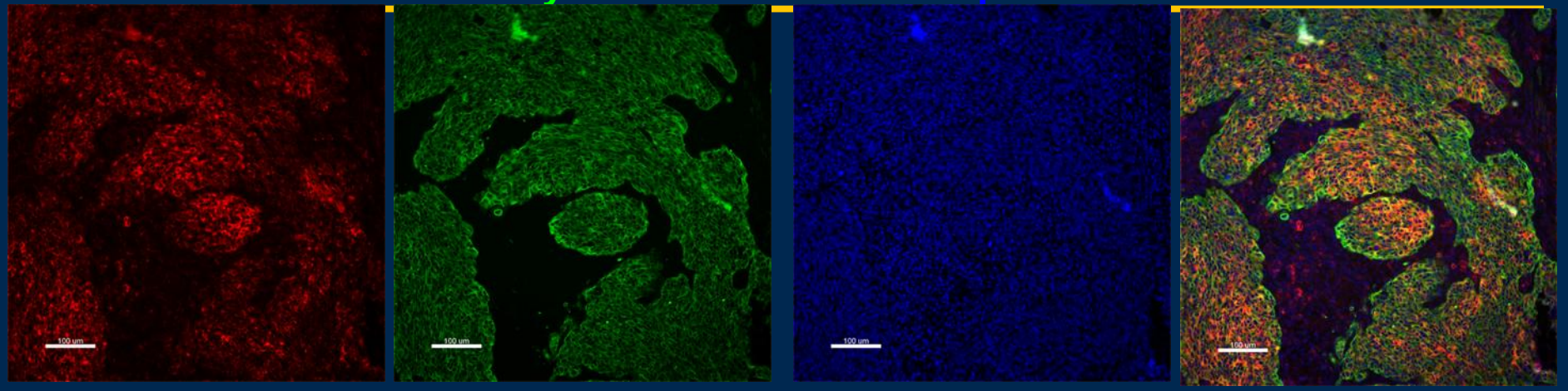
Ovarian Papillary serous CA – High Expression in Epithelial component



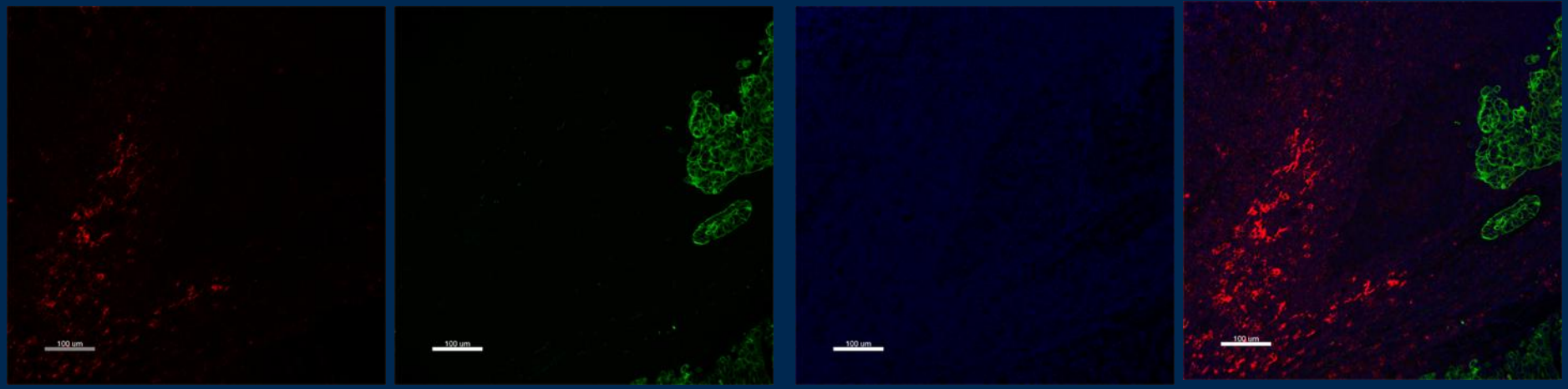
Ovarian Endometrioid CA – High Expression in Stroma

TRAIL Staining in Ovarian Tumor

TRAIL | Cytokeratin | Dapi | Combination



Ovarian Serous CA – High Expression in Epithelial Components



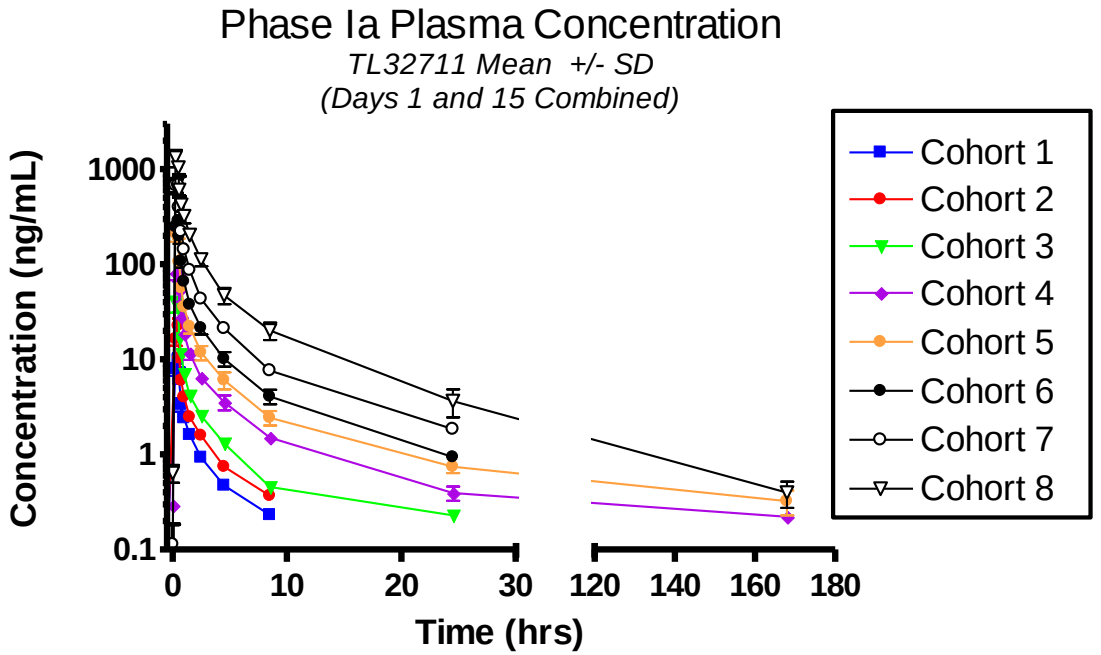
Ovarian Papillary Serous CA – Stroma Expression only

TL32711 Single Agent Phase 1 Study Dose Escalation Status

Cohort No.	Dose (mg/m^2)	% Increase from Prior Dose	C_{max} (ng/ml)	AUC (ng.h/mL)	cIAP1 Supp at 24hrs	cIAP1 Supp at 7 days
1	0.18		10	11	25-50%	0%
2	0.36	100%	22	20	25-50%	0%
3	0.72	100%	41	37	25-50%	20%
4	1.44	100%	86	114	>75%	50%
5	2.88	100%	258	254	>75%	>50%
6	5.76	100%	293	329	>75%	>50%
7	11.5	100%	664	669	>85%*	>60%*
8	17.2	50%	1338	1657	>85%*	>75%*
9	26	50%	2000 (est)	2500 (est)	Pending	Pending

TL32711 Pharmacokinetics

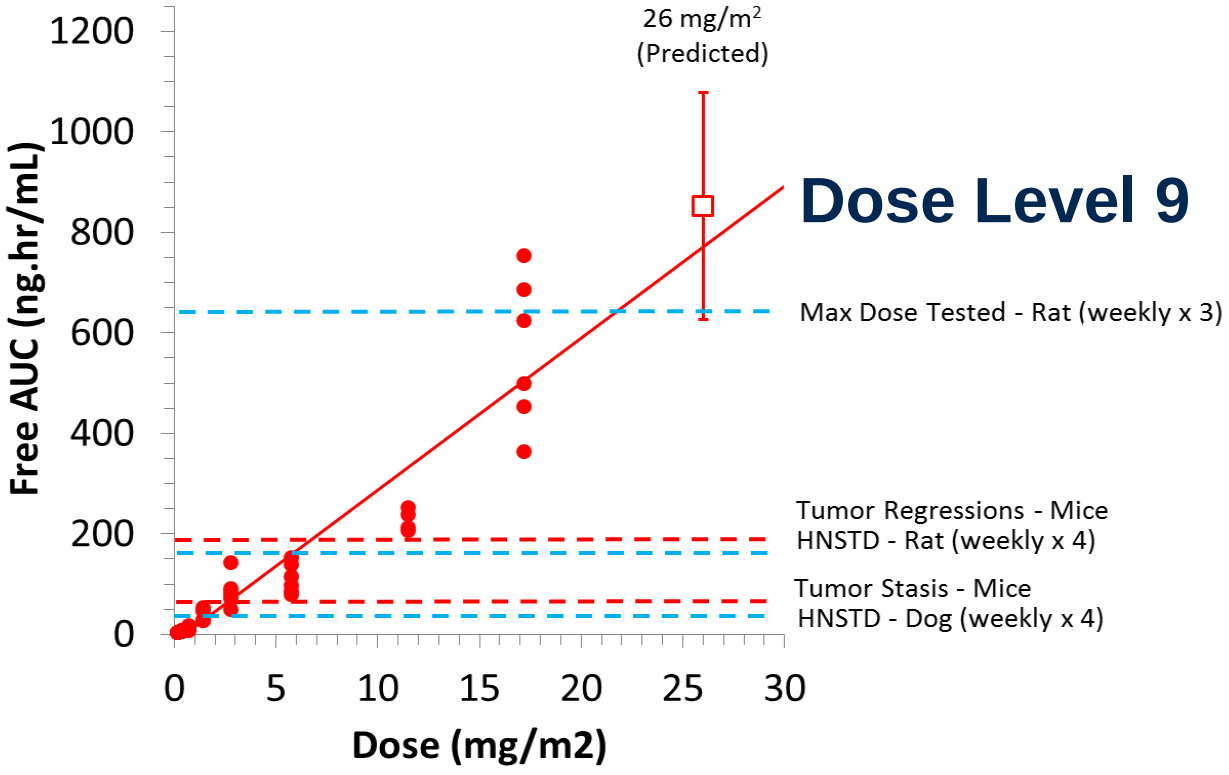
(Cohorts 1 through 8 Total Drug Levels)



Dose (mg/m²)	0.18	0.36	0.72	1.44	2.88	5.76	11.5	17.2
Cmax (ng/mL)	9.9	22.4	41.3	86.1	258.2	292.5	664.5	1338
AUC (ng.h/mL)	10.5	20.0	37.3	113.6	254.3	328.7	669.3	1657
$t_{1/2}$ (h)	1.9	2.5	3.5	6.7*	7.1*	6.3	6.1	34.6

*Excludes 3 subjects (02-402, 03-501 and 01-502) with long terminal t1/2 values (73-87 h)

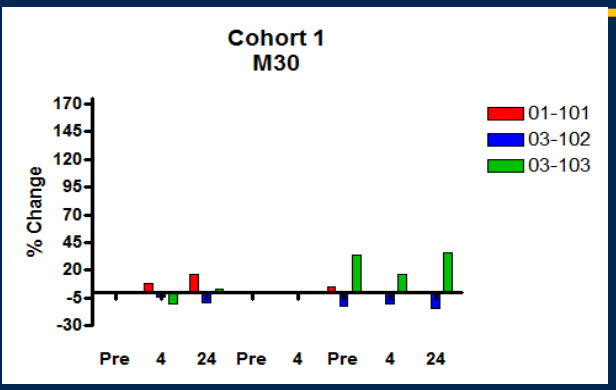
TL32711 Clinical Pharmacokinetics

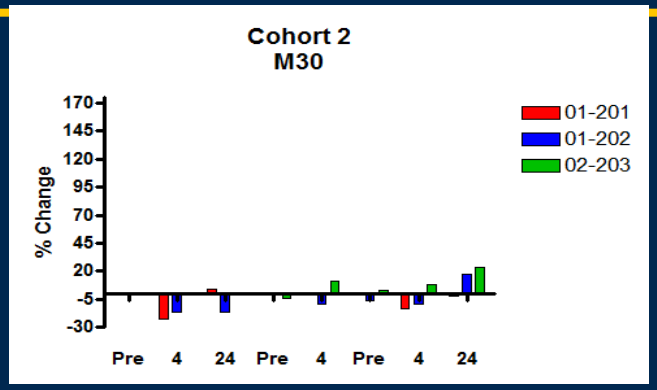


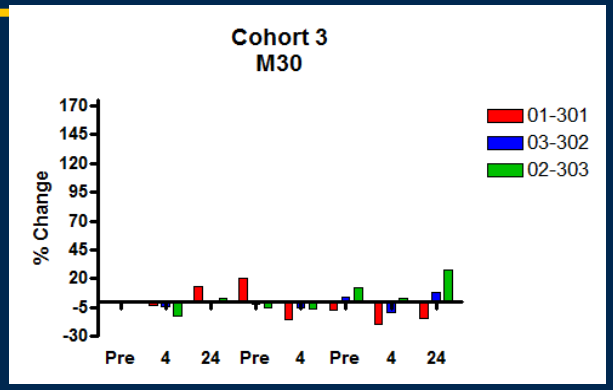
(No toxicities)

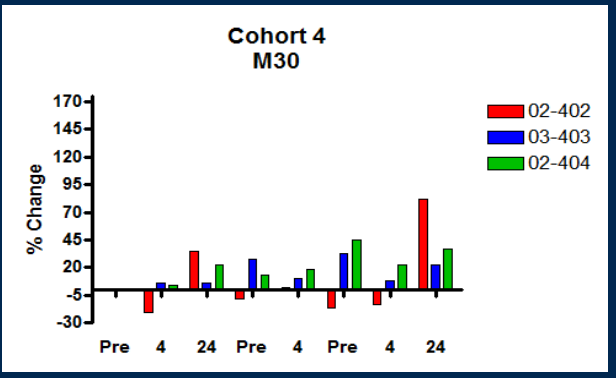
Apoptosis Pathway Activation by TL32711

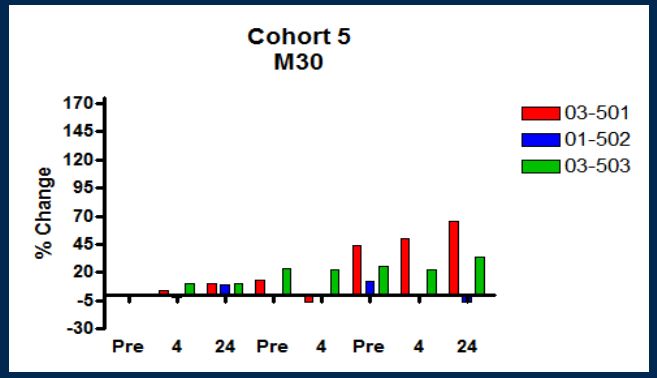
Caspase-3 dependent Cleaved Cytokeratin-18 Serum Levels

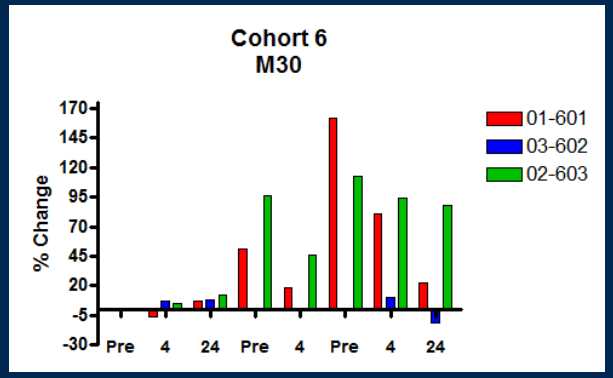


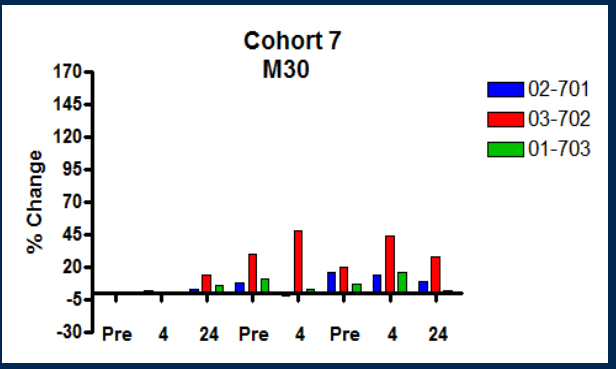


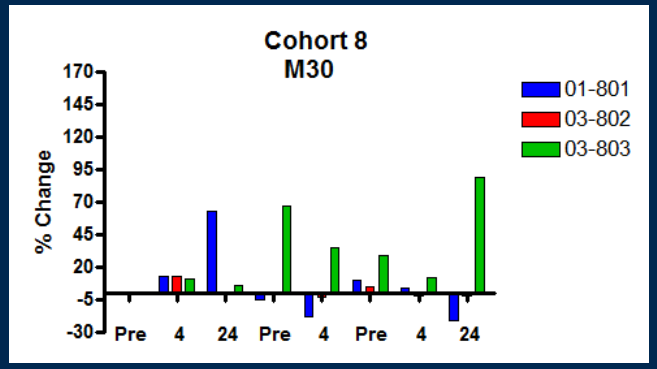






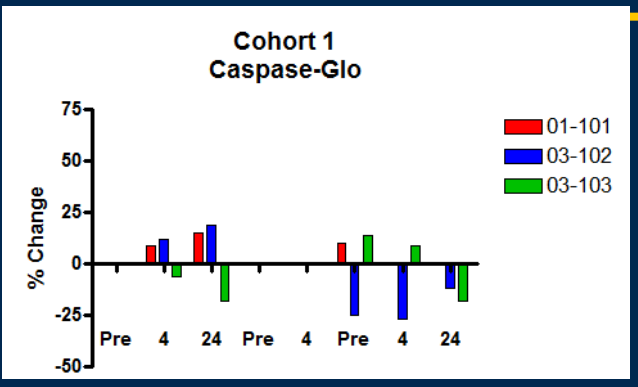


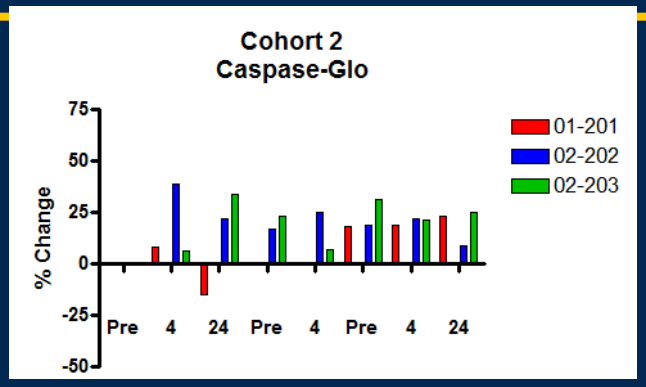


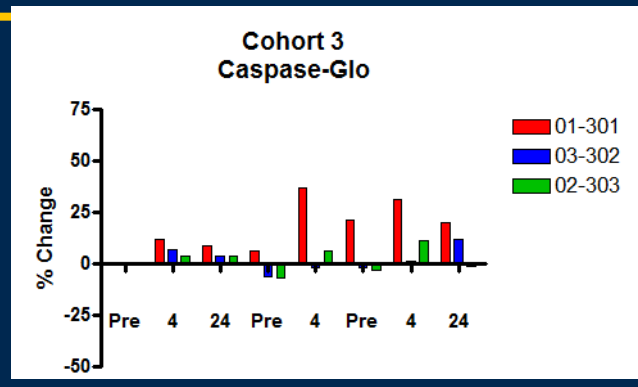


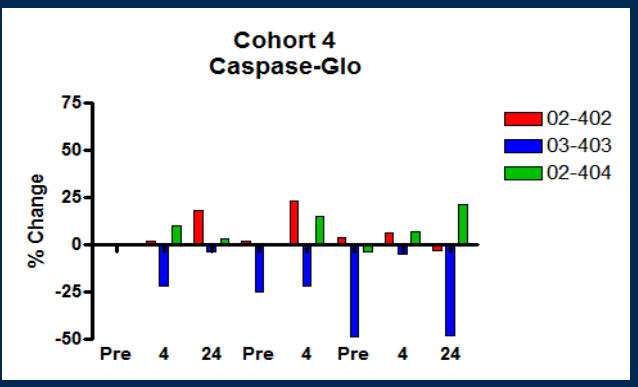
Apoptosis Pathway Activation by TL32711

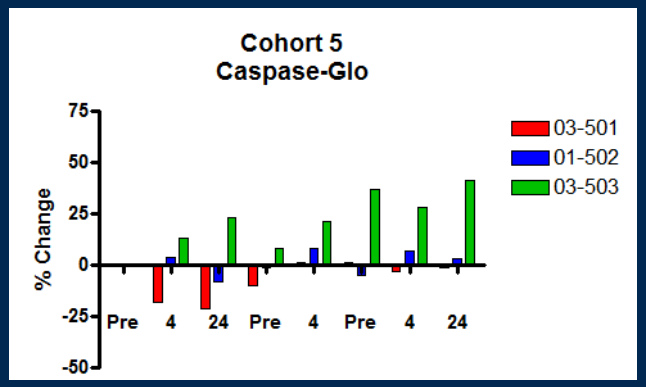
Activated Caspase-3/7 Serum Levels

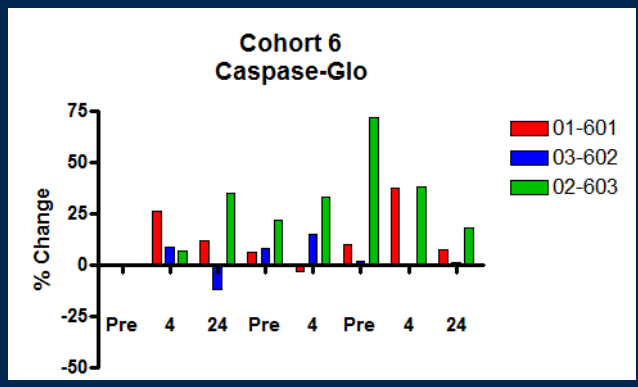


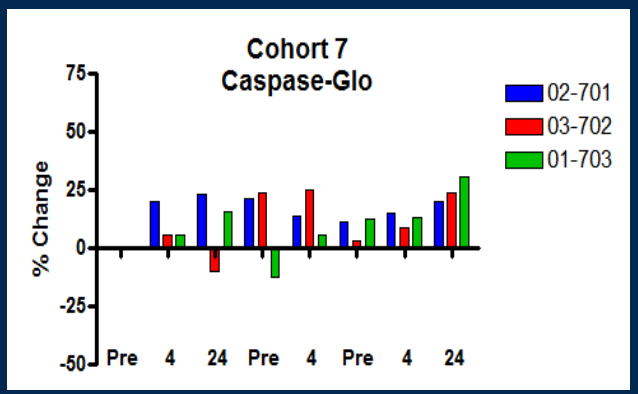


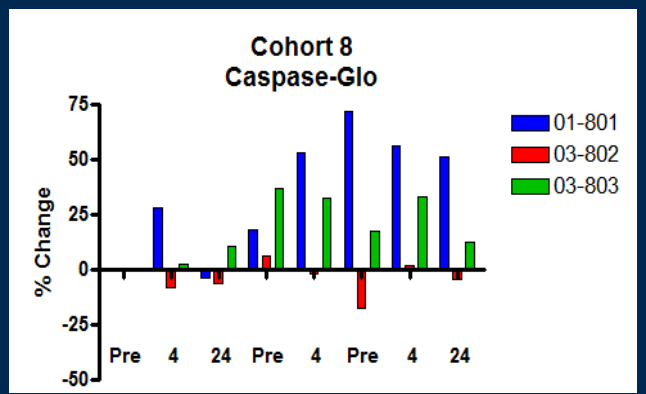






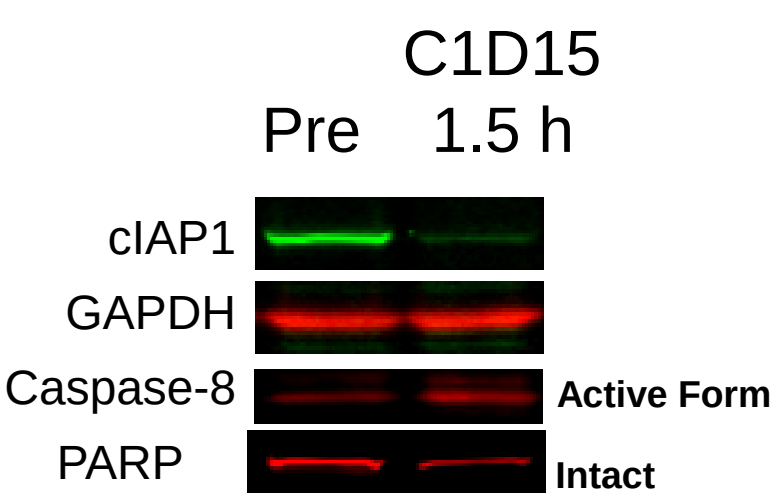


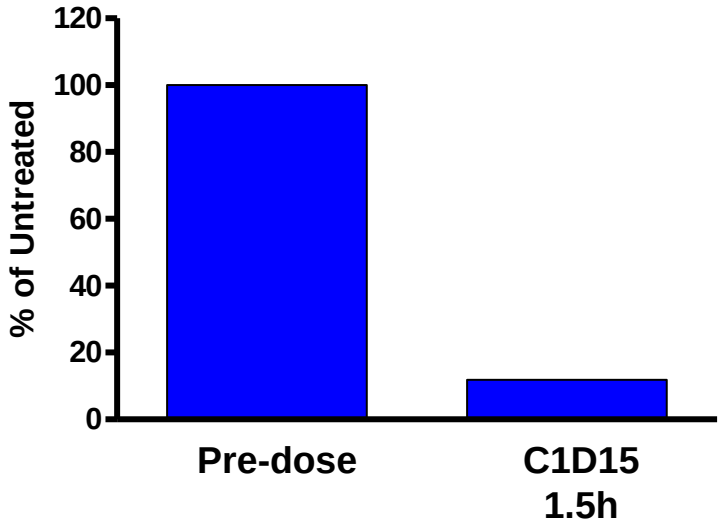




Degradation of cIAP1 and Apoptosis Pathway Activation in Tumor Biopsy

01-703 Biopsy Western Blot Analysis





cIAP1 degraded by ~90% after TL32711 treatment
Activated caspase-8 seen
Intensity of intact PARP lower suggesting cleavage by Western

Phase 1 Clinical Comparison

TL32711		LCL161	HGSI1029
Dose Level	**Bio-Effect**	**Dose Level**	**Dose Level**
		1 to 5 (10/20/40/80/160 mg/pt)	1 (0.1 mg/m^2) - cIAP-1 suppression
1 (0.18mg/m^2)	•cIAP-1 suppression (50-60%) •IL-6 suppression •No cytokine elevation •No AEs	6 (320 mg/pt) -cIAP-1 suppression -M30 and M65 increases -no tox	2 (0.2 mg/m^2) •cIAP-1 suppression
2 (0.36mg/m^2)	•cIAP-1 suppression (70-75%) •Elevation of activated caspase-3 activity in serum •No cytokine elevation •No AEs	7 (500 mg/pt)	3 (0.4 mg/m^2) Grade 1/2 AEs •Nausea •Diarrhea •Anorexia •Vomiting •fatigue •Pyrexia •Dose-dependent lymphocytopenia •Transient neutrophilia
3 (0.72 mg/m^2)	•cIAP-1 suppression (80-90%) •3 subjects without AEs •No cytokine elevation •No significant inc for casp-3 or CK levels	8 (900 mg/pt) •Dose-related increased cytokines •High prevalence of N/V	4 (0.6 mg/m^2) •Grade 3 INR •Grade 3 Supraventricular Tach •Grade 3/4 lymphocytopenia
		9 (1800 mg/pt) Grade 3/4 - N/V	5 (0.9 mg/m^2) •cIAP-1 suppression (80-90%)
4 (1.4 mg/m^2)	•cIAP-1 suppression (80-90%) and prolonged •Well tolerated	10 (2400 mg/pt) -Capsule formulation ongoing	6, (1.4 mg/m^2) •cIAP-1 suppression (80-90%) •1 DLT (AST G3, Amylase G3, Lipase G4, Fatigue G3)
5 (2.8.mg/m^2)	•Well tolerated		7 (2.1 mg/m^2), 8 (3.2 mg/m^2) •cIAP-1 suppression (80-90%)

TL32711 Phase 1B Multi-Arm Combination Trial

1 – Carboolatin/Paclitaxel (AUC -6, 175mg/m^2, q3 weeks)

2 – Irinotecan (350 mg/m^2 q3 weeks)

3 – Docetaxel (75 mg/m^2 q3 weeks)

4 – Gemcitabine (1000mg/m^2 qweek x 3 of 4 weeks)

5 - Liposomal Doxorubicin (40 mg/m^2 q4 weeks)

Summary

- Alterations in IAPs are found in many cancers and are associated with chemoresistance, disease progression and poor prognosis
- SMAC mimetics inhibit IAPs
- Cancer cells are very sensitive to SMAC mimetics in the presence of TNF alpha
- SMAC mimetics synergize with TRAIL and multiple chemotherapy agents
- Single agent activity is expected in tumors with secretion of TNF alpha in microenvironment
- Combinations with chemotherapy are expected to be efficacious

THANKS !

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