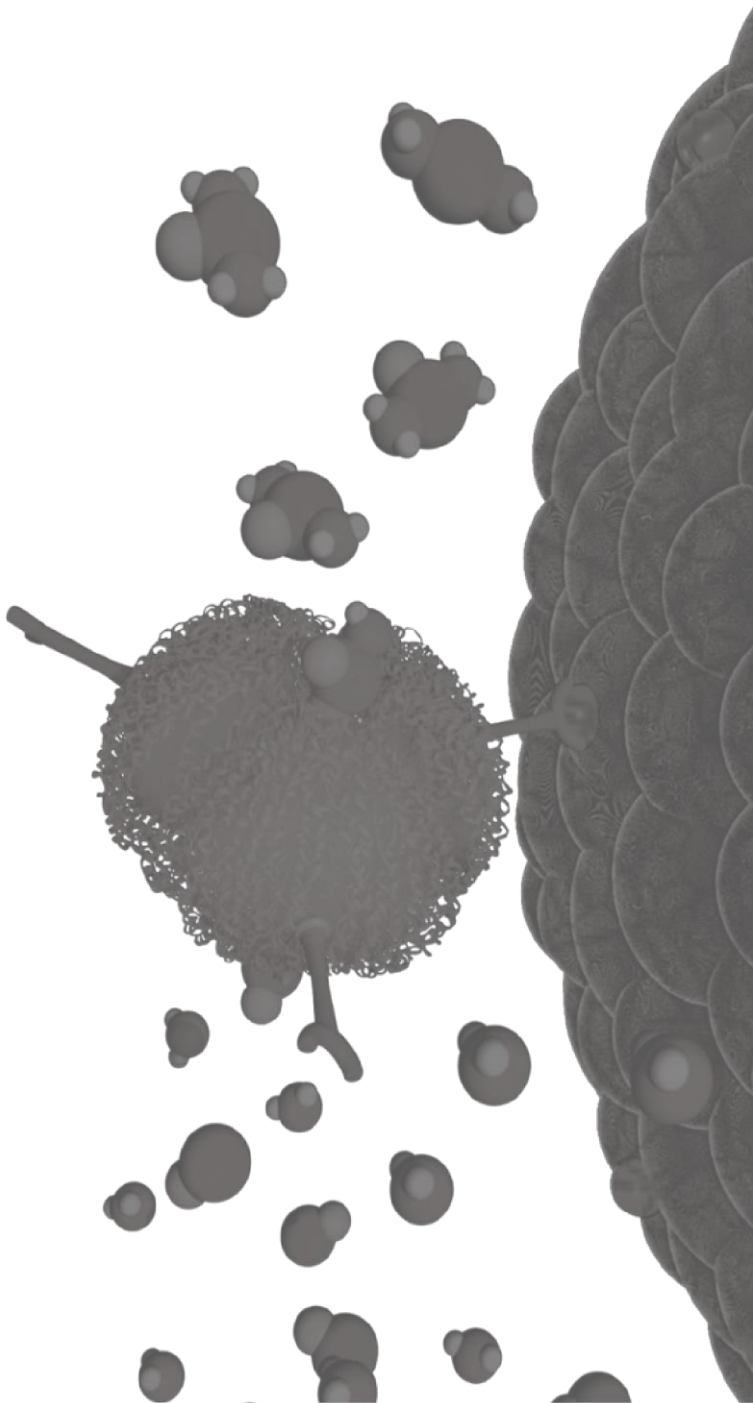
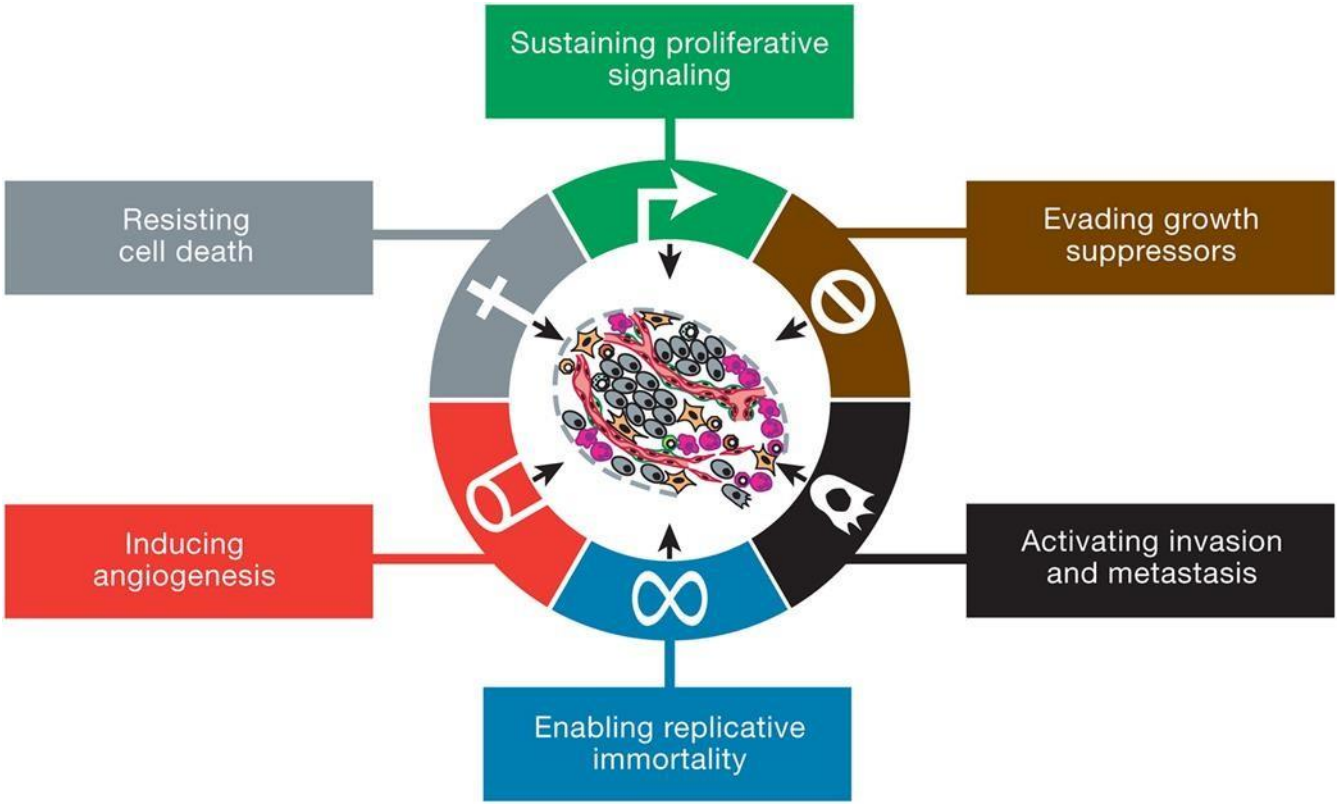
HelixBioPharmaCorp.

Therapeutic Strategy Against Tumour Acidity Induced Immune-Suppression



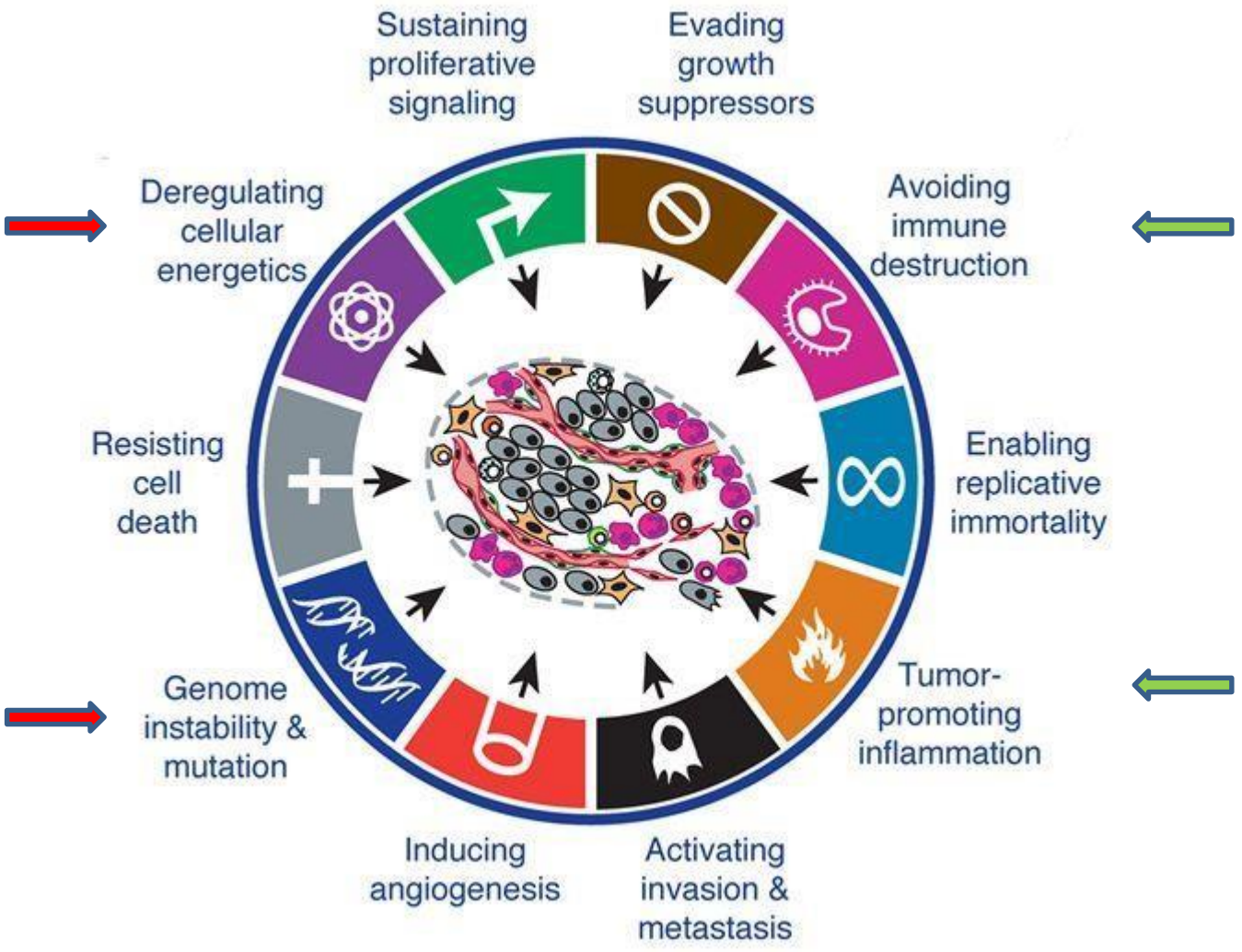
STREAM Summer School
Medical University of Warsaw
June 2016

The Hallmarks of Cancer



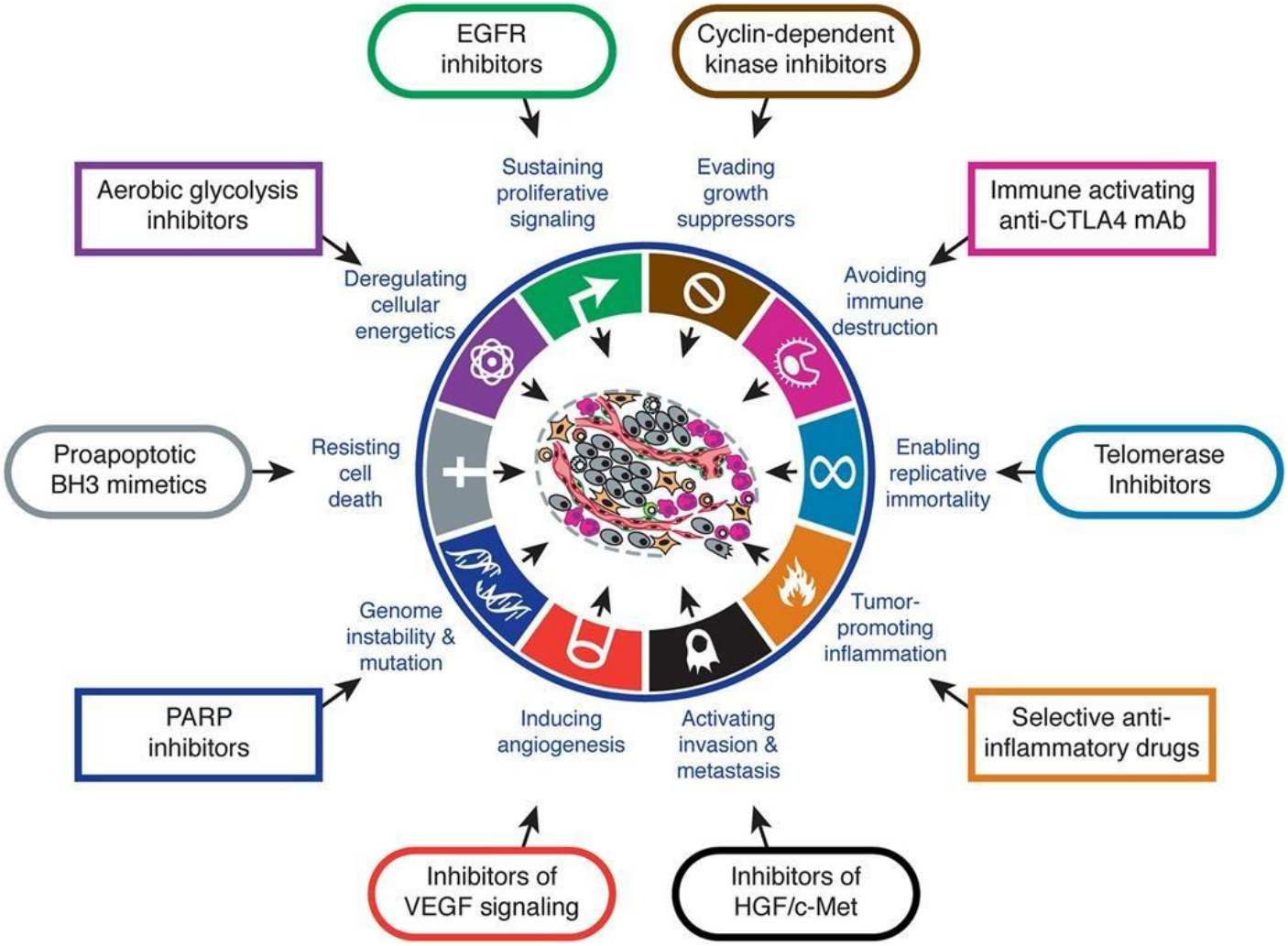
Cell 2011 144, 646-674

The Hallmarks of Cancer: Next Generation



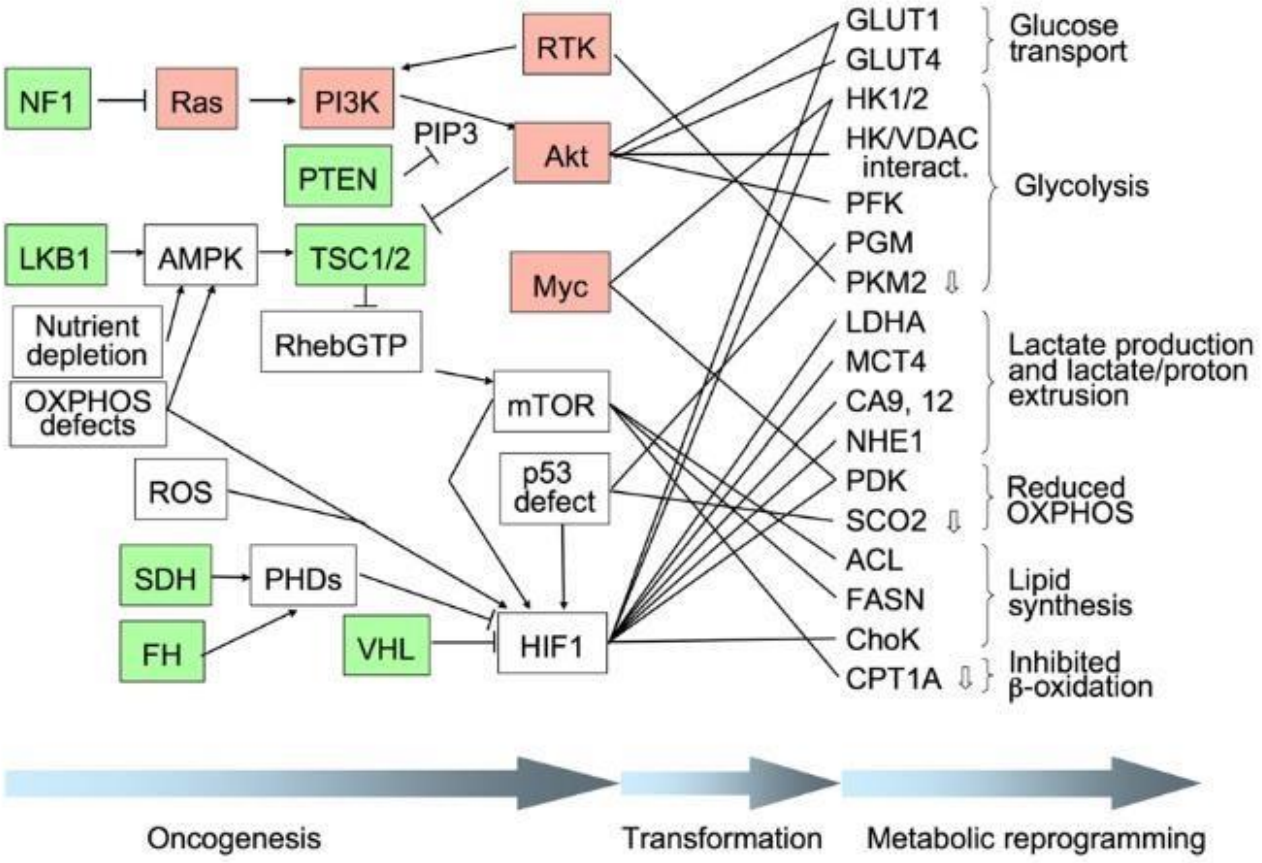
Cell 2011 144, 646-674

Treatment Strategy



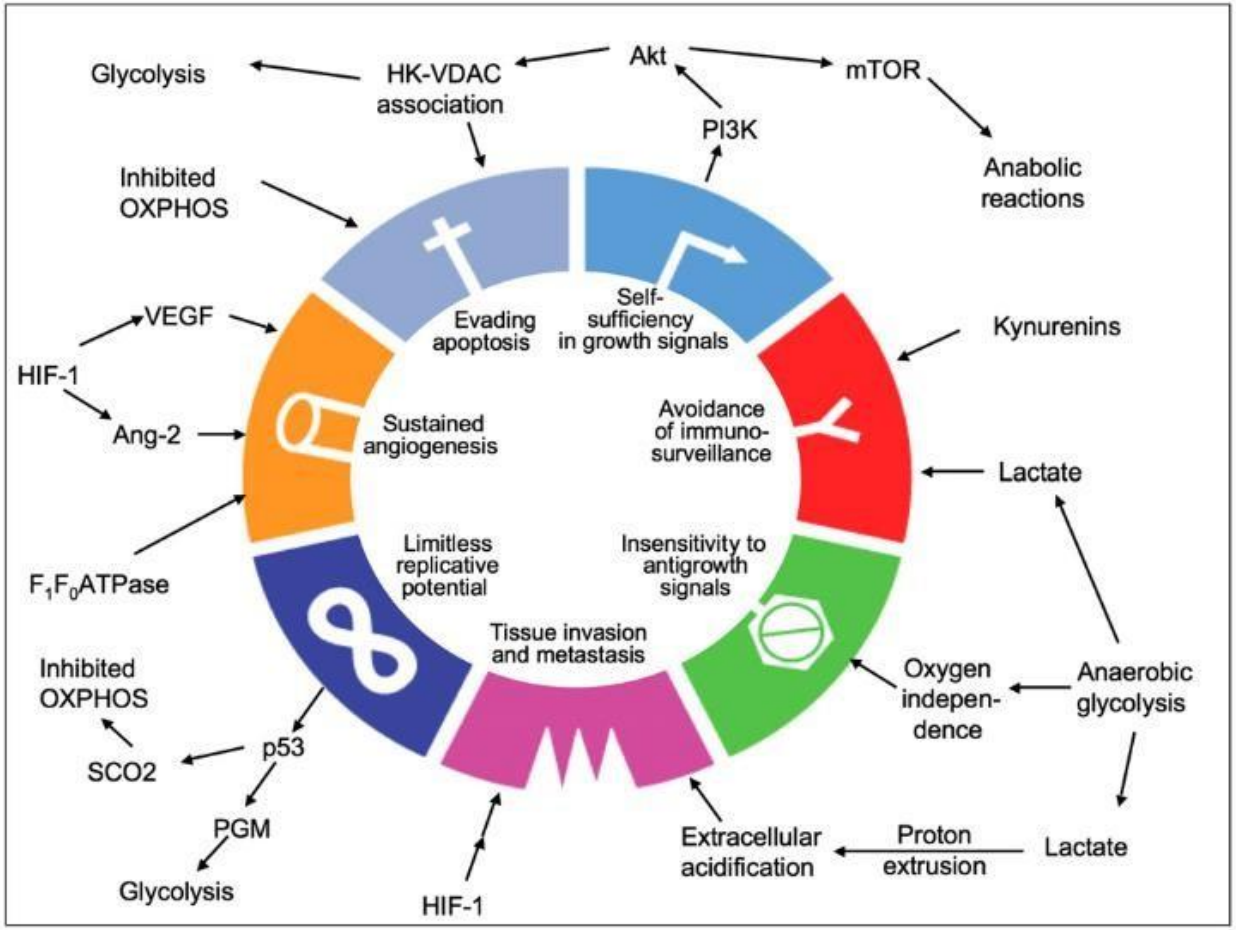
Cell 2011 144, 646-674

Metabolic Reprogramming



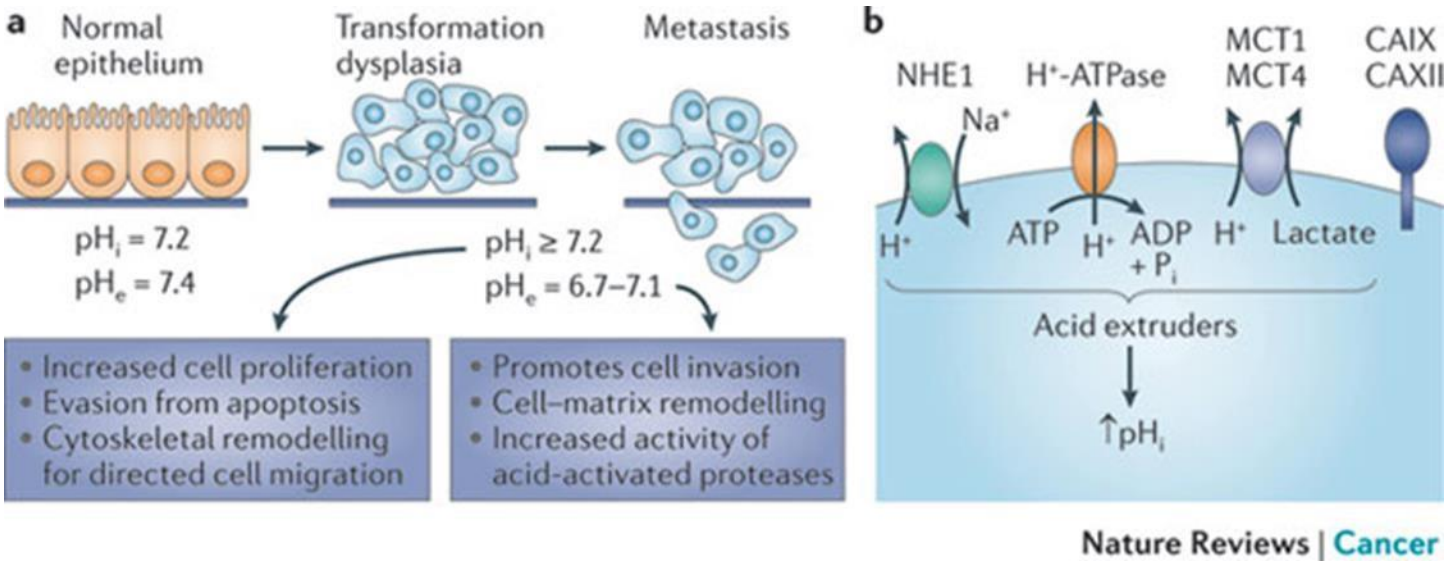
Cancer Cell 2008, 13 472-482

Cancer Hall Marks Link to Metabolism



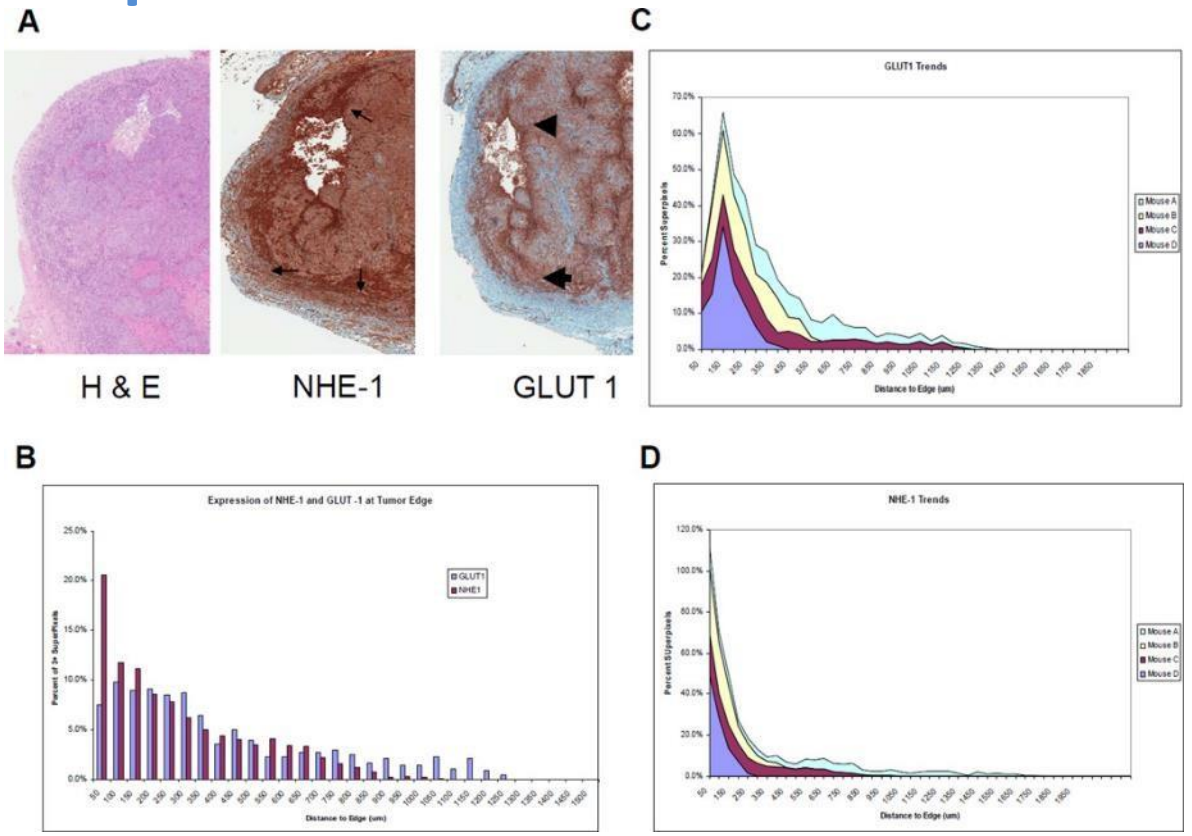
Cancer Cell 2008, 13 472-482

Dysregulated pH is Emerging as a Hallmark of Cancer



Nature Reviews Cancer 11, 671-677 (September 2011)

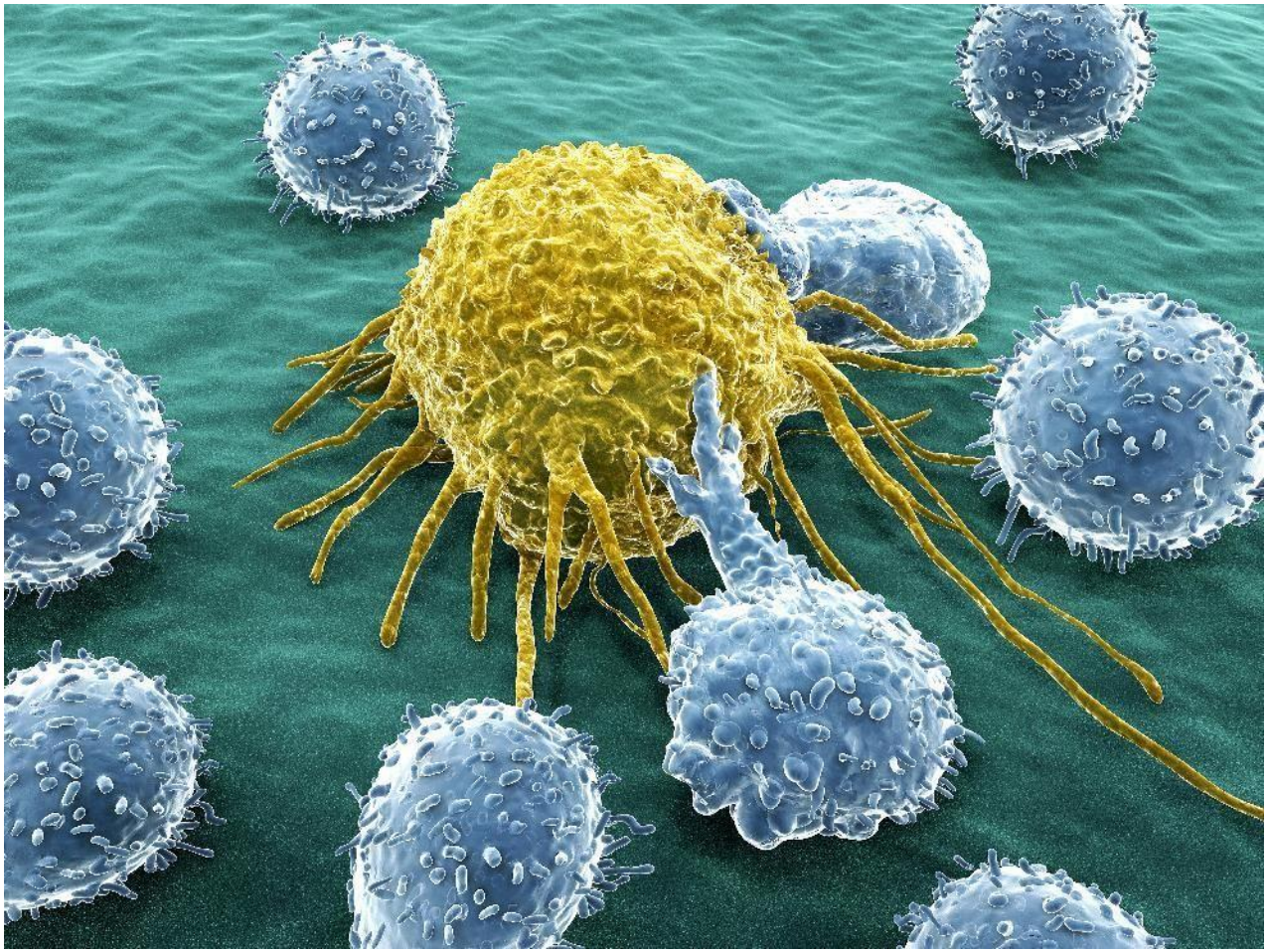
Low pH and Tumor Invasion



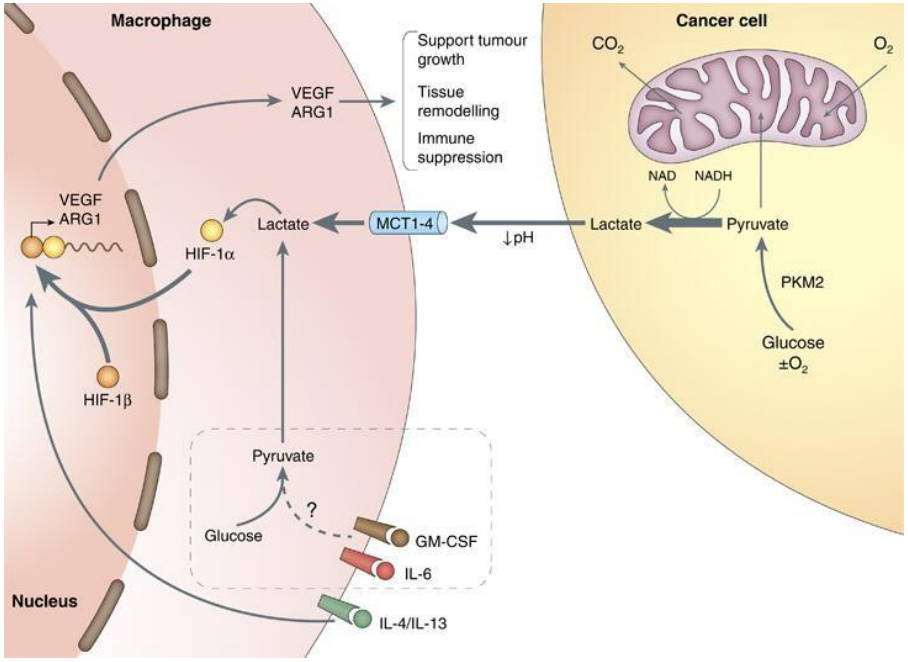
(A) The tumor edge has an increased expression of NHE-1 (small thin arrows) and GLUT1 (large arrows), which is indicative of acidification caused by an increase in glycolysis. This is consistent with microenvironmental acidosis observed in vivo leading to subsequent invasion. (B) Expression of GLUT-1 and NHE-1 as a function of distance from the tumor edge. (C) and (D) Expression trends of GLUT-1 and NHE-1 as a function of distance from tumor edge in N=4 tumors.

Cancer Res. 2013 Mar 1; 73(5): 1524–1535

Tumor pH Effects on Immune Cells



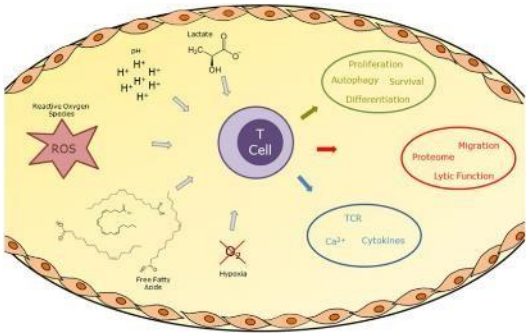
Lactate Lower Tumour pH and Polarize Macrophages



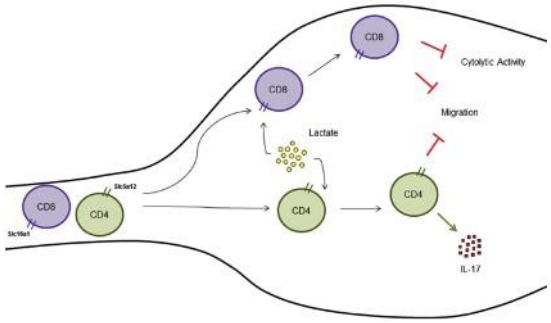
Colegio et al. Nature 513:559-563 (2014)

Macrophages integrate metabolic and environmental signals to promote tumor growth. Tumor lactate which lower pH polarizes macrophage and up-regulate Arg1. Area within dotted rectangle indicates proposed mechanisms of action. ARG, arginase; HIF, hypoxia-inducible factor; MCT, monocarboxylate transporter; NADH, nicotine adenine dinucleotide, reduced; PKM2, M2 isoform of pyruvate kinase; VEGF, vascular endothelial growth factor.

T Cell Loss of Function from Low pH and Lactate



Haas et al. Am J. Clinic. Immunol. 2(2): 146-155 (2013

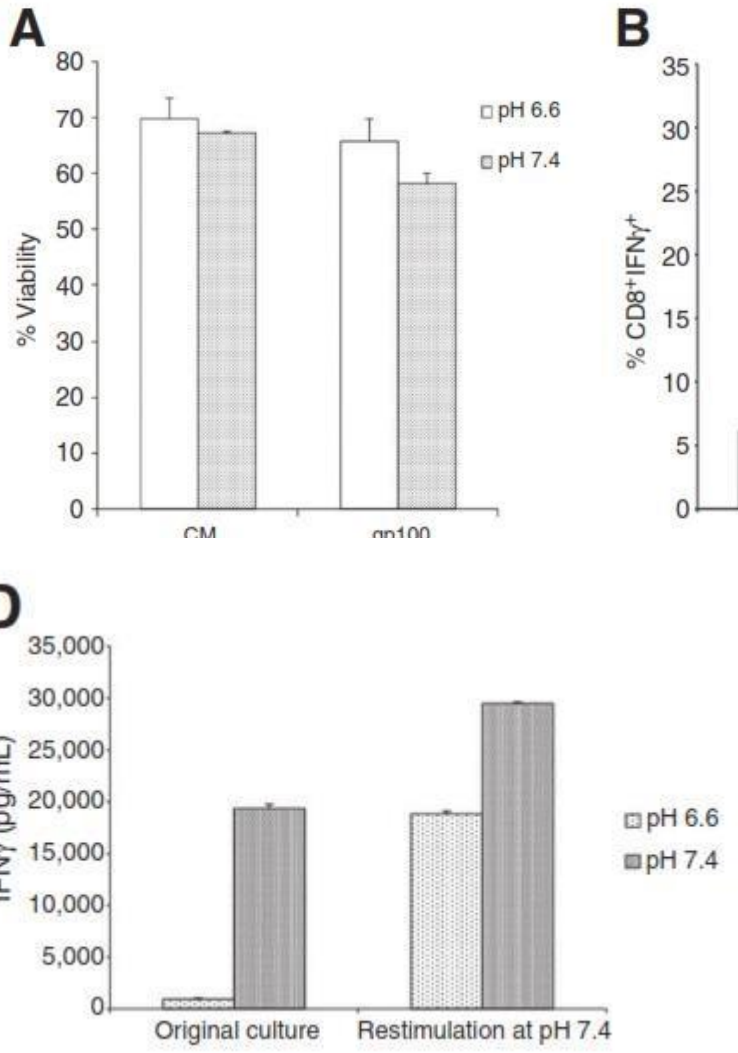


Haas et al. PLoS Biol. 13(7) (2015)

Several factors in the inflammatory microenvironment (e.g., oxygen concentration, pH, lactate, fatty acids and ROS) can influence the function of T cells and other immune cells on a number of levels and determine the outcomes of the inflammatory process.

The motility of CD4+ and CD8+ T cells is blocked once they get exposed to elevated levels of lactate in the inflammatory site. Lactic acid also causes loss of cytolytic activity by CD8+ T cells, and sodium lactate promotes the production of IL-17 by CD4+ T cells.

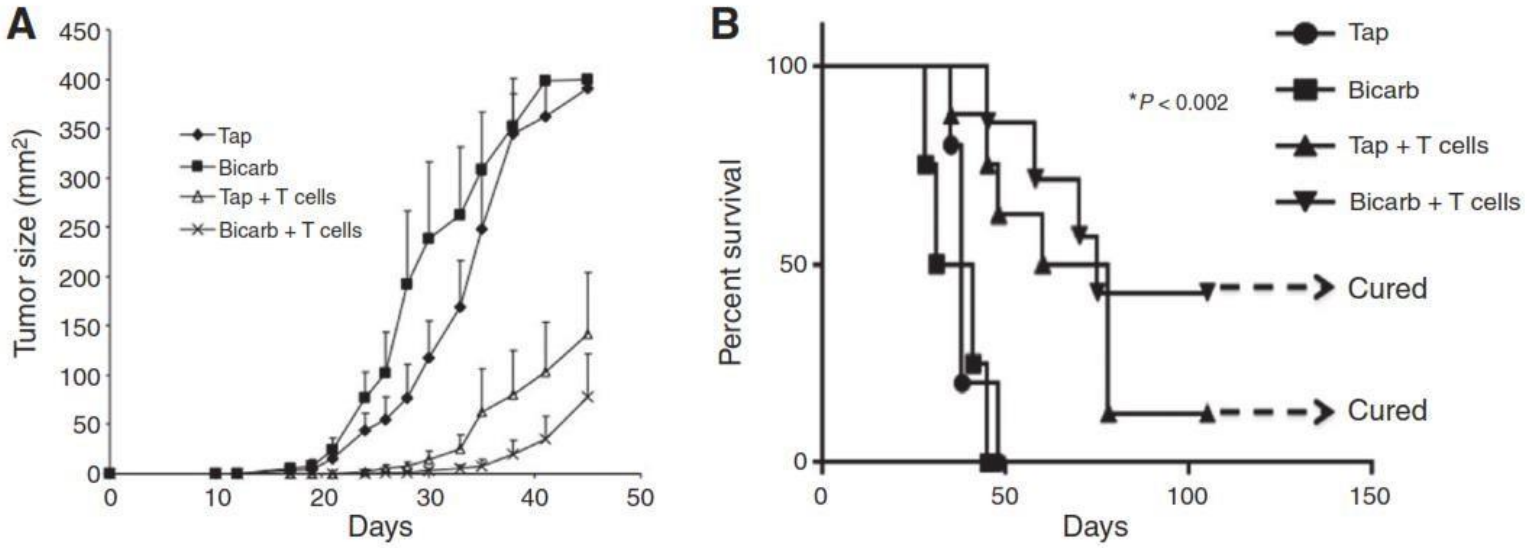
T Cell Loss of Function from Low pH



CD8+ T cells isolated from pmel mice transgenically express a TCR specific for the melanocyte-associated peptide, Gp100(25-33) while remain viable in low pH, has lower IFNg secretion upon stimulation (A,B). The effect can be reversed by re-stimulation in higher pH (D).

Cancer Res; 76(6) March 15, 2016

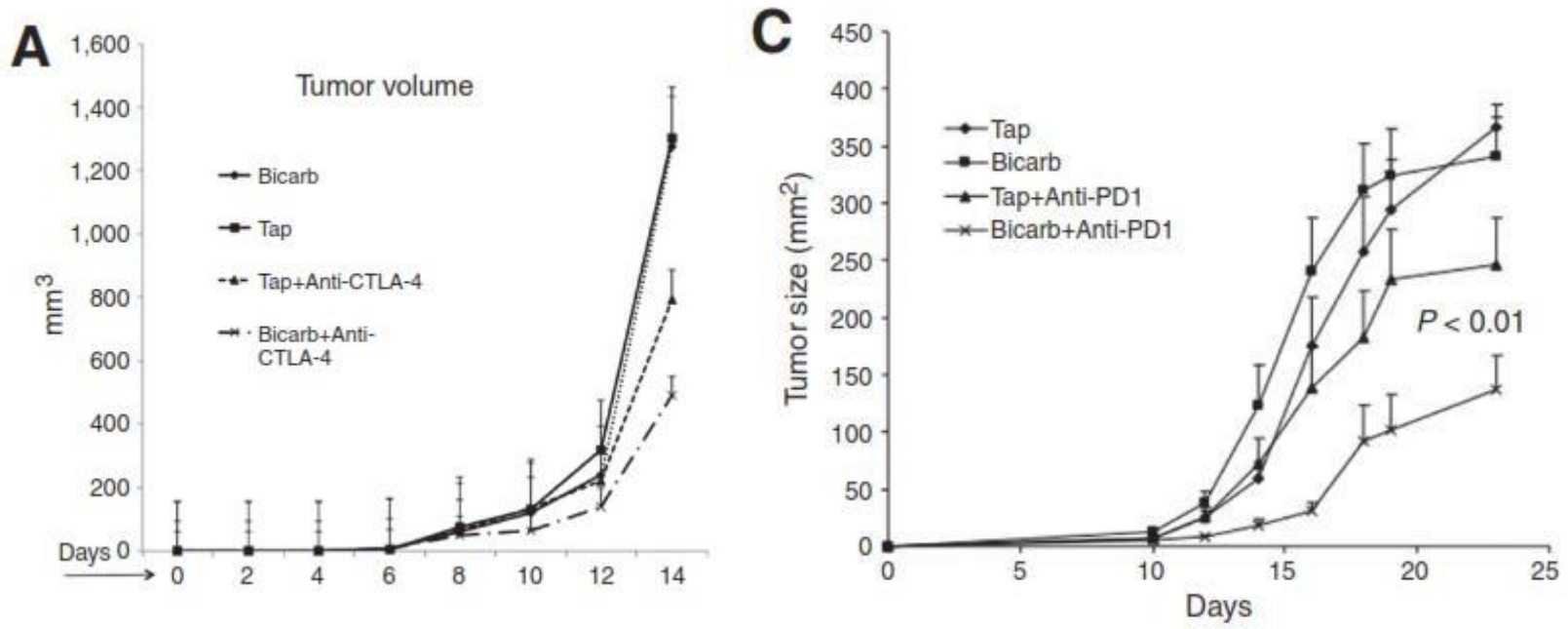
Acidity Affects Adoptive T cell Therapy



Effect of bicarbonate on adoptive T-cell transfer. A, tumor growth after adoptive transfer of T cells or controls in combination with or without buffer therapy. Group mean differences between T cells vs. T cells vs bicarbonate were not significant. However, there was a survival advantage, as shown in the survival curve in B

Cancer Res; 76(6) March 15, 2016

Tumor pH and Check-Point Inhibitors

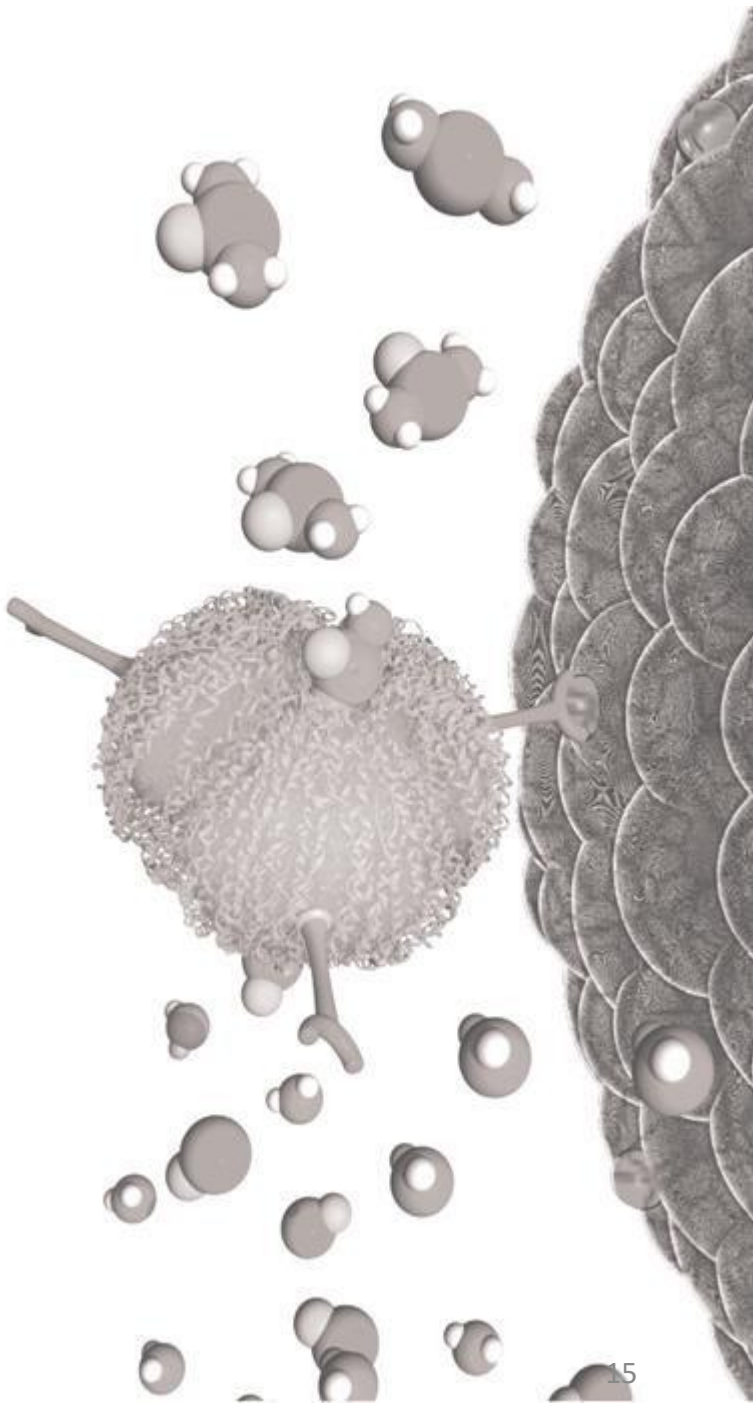


Buffer therapy enhances efficacy of anti-immunotherapy in B16 melanoma. C57BL/6

Cancer Res; 76(6) March 15, 2016

Targeting the tumor microenvironment

DOS47

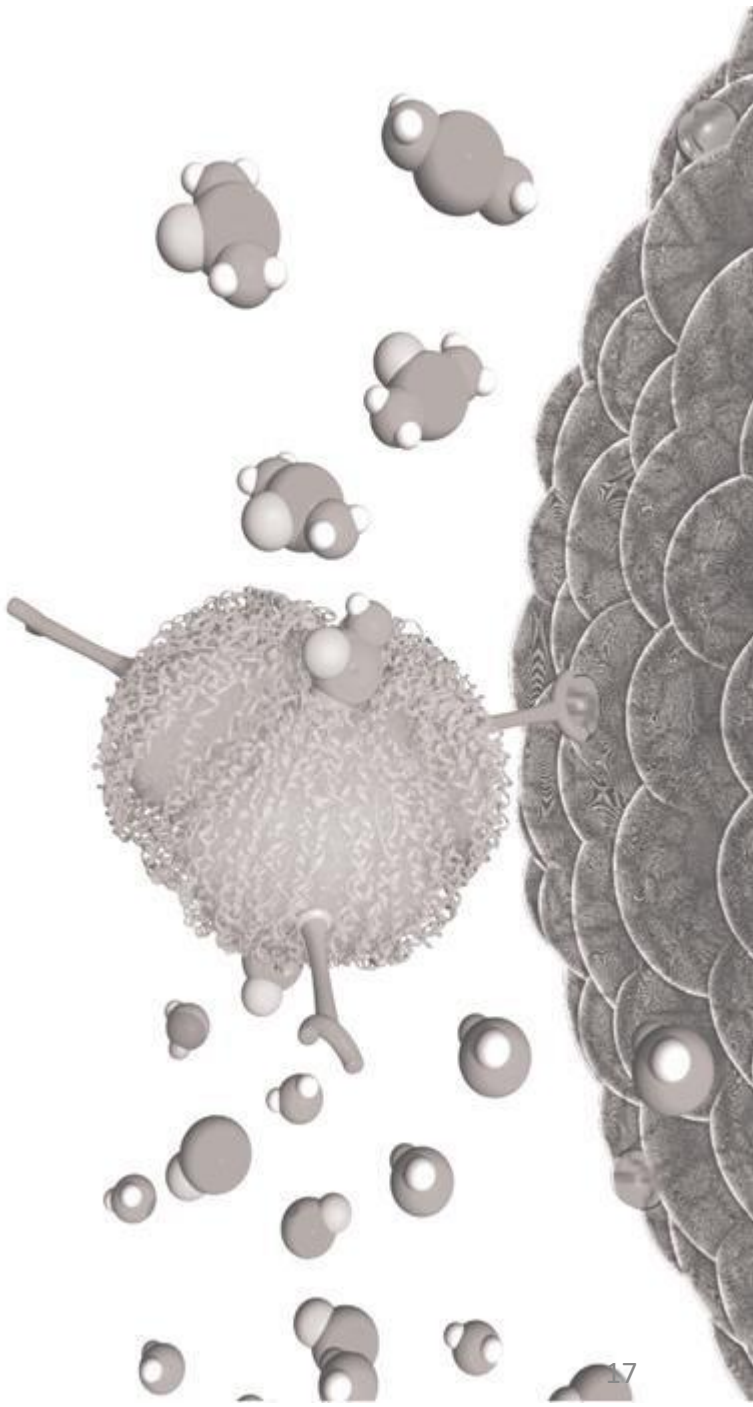


DOS47

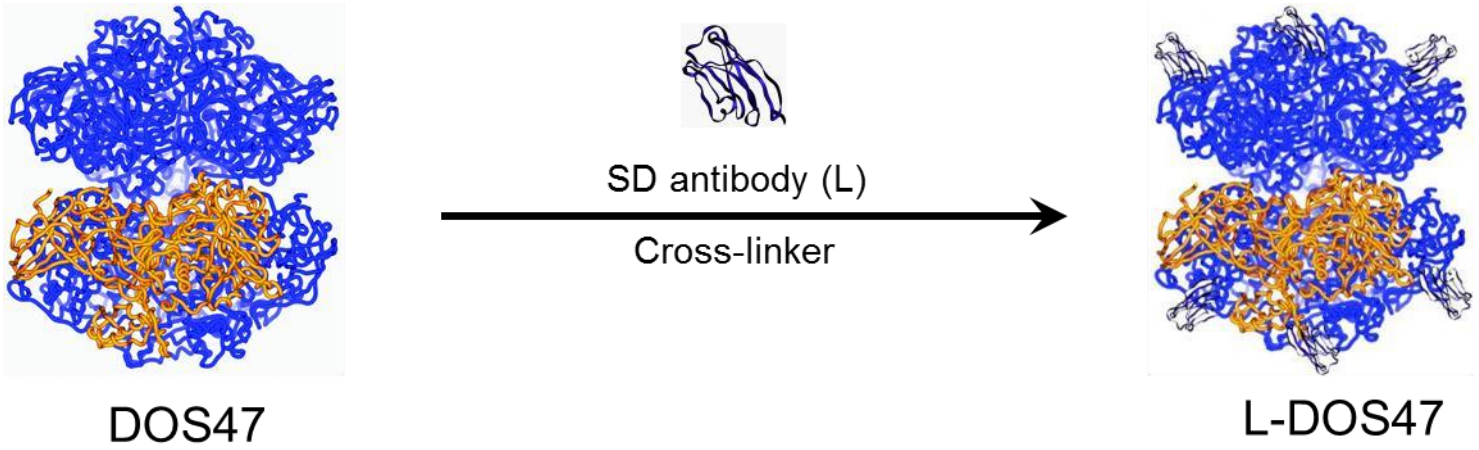
- DOS47 is a technology that changes the tumor microenvironment from acidic to alkaline using the enzyme ‘urease’
- Alkalinizing the tumor has the potential to
  - To exert direct cytotoxic effect on tumours
  - to increase the action of certain chemo-therapies
  - to correct an impaired immune microenvironment

Helix First Clinical Drug Candidate

L-DOS47



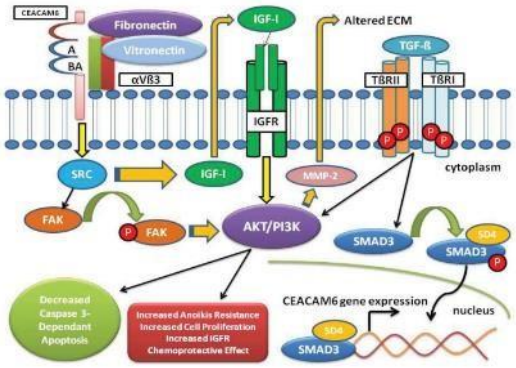
L-DOS47



- L-DOS47 is a conjugate of urease with a proprietary camelid single domain antibody specific for CEACAM6
- CEACAM6 is a cell surface tumor antigen highly expressed on lung, colon, pancreatic and other cancer cells
- L-DOS47 is in clinical studies for the treatment of non-squamous, non-small cell lung cancer (NSCLC)

L-DOS47 – Dual Function

Antigen: CEACAM6

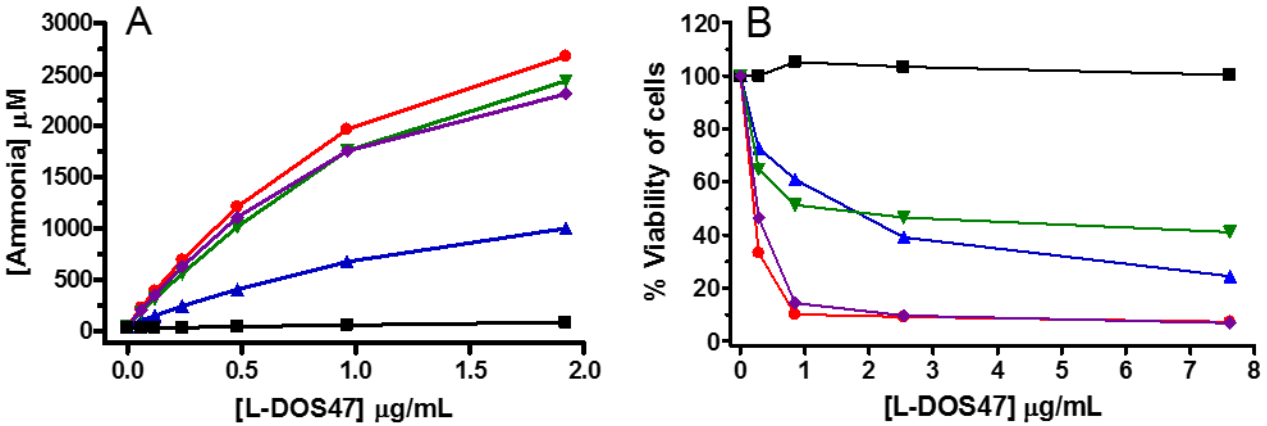


- Glycosylated 90 kDa (286aa) GPI-linked membrane protein
- Intercellular adhesion molecule forming homotypic and heterotypic bonds with CEACAM-1, 5 and -8
- Important for cell attachment and proliferation
- May act as a checkpoint inhibitor in Multiple Myeloma

Enzyme substrate: urea

- Urea is a natural metabolite
- Ammonia/ Ammonium produced from urea hydrolysis is toxic to cells
- Apoptotic enzymes caspase 2 and 3 (A549 lung cell) and caspase 8 and 9 (BxPC3 pancreatic cells) are induced

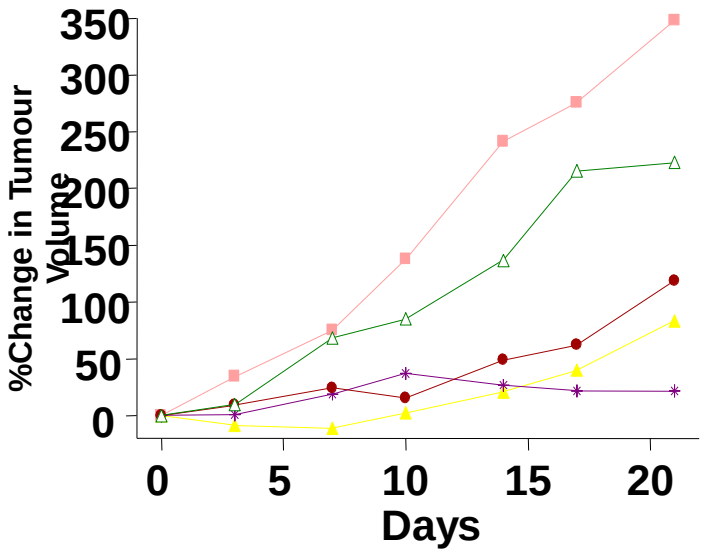
L-DOS47 Cytotoxic to CEACAM6 cells



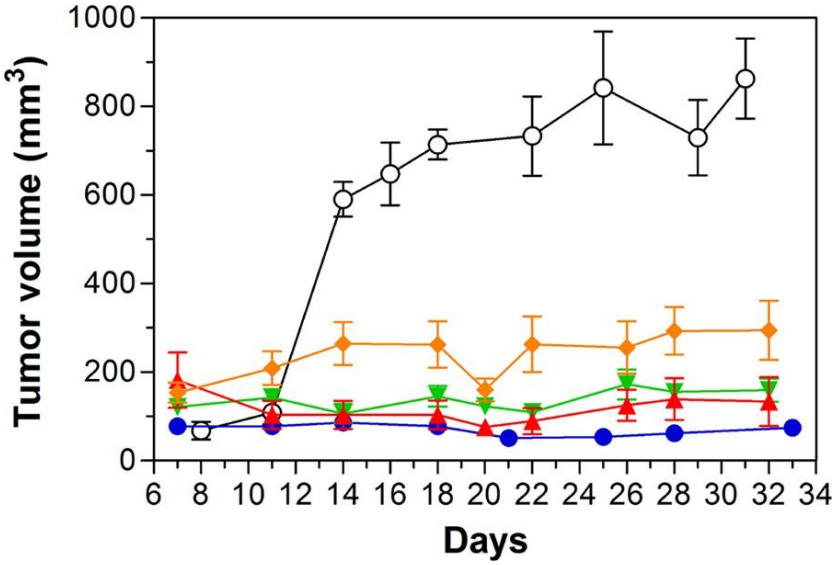
Cell lines		Binding assay	Cytotoxicity assay
MDA-MB231	Breast adenocarcinoma	-	-
MCF-7	Breast carcinoma	-	-
ZR-75-30	Breast ductal carcinoma	+++	+++
LS174T	Colon adenocarcinoma	++	++
A549	Lung adenocarcinoma	++	+
H23	Lung adenocarcinoma	-	+
BxPC-3	Pancreatic adenocarcinoma	+++	+++
Capan-1	Pancreatic adenocarcinoma	+++	++
MIA PaCa-2	Pancreatic carcinoma	+	+

Where: +, positive (the number of + indicates the strength of activity); -, negative

L-DOS47 Inhibits Tumor Growth in Lung and Pancreatic Models



A549 (lung)
L-DOS47 (10,20,35U/kg)
Cisplatin control



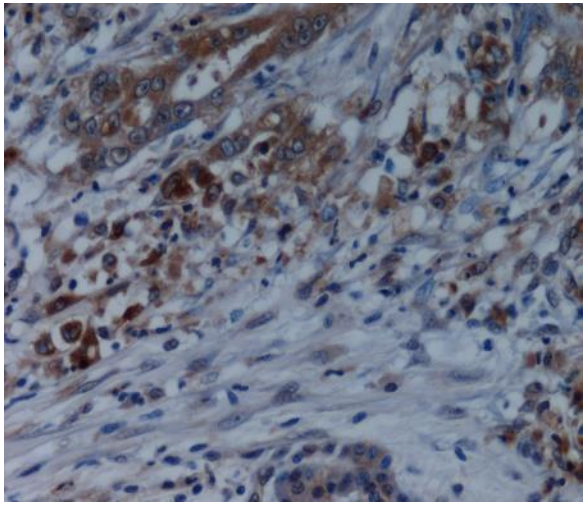
BxPC3 (Pancreatic)
L-DOS47 (7,35,175ug/kg)
Paclitaxel control

Tian et. al. Bioconjug Chem. 2015 Jun 17;26(6):1144-55

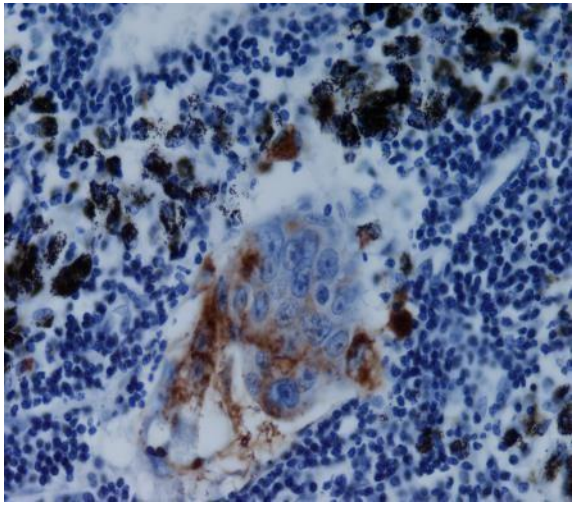
L-DOS47 Binds to CEACAM6 Positive Cancer Patient Tissues

Samples	Tumour Tissue		Age-matched Normal Tissue
	Positive	Negative	Negative
Kidney carcinoma		12/12	12/12
Parathyroid adenoma		1/1	n/a
Plaenta, umbilical cord, allantois	n/a		1/1
Myofibroblastic tumor		1/1	n/a
Prostate carcinoma		4/4	4/4
Thyroid carcinoma		2/2	2/2
Pancreas adenocarcinoma	7/57 weak 8/57 v. weak	42/57	25/25
Neuroendocrine tumors		9/9	n/a
Brain, heart muscle, testis, spleen	n/a		30/30
Testis - teratoma and seminoma		3/3	3/3
Parotis tumor		1/1	1/1
Cervix squamous carcinoma		2/2	n/a
Thymoma		2/2	n/a
Colon adenocarcinoma	14/24 weak	10/24	24/24
- lymph node metastasis		3/3	
Breast adenocarcinoma		13/13	13/13
- lymph node metastasis		2/2	
Leiomoma - lung metastasis		1/1	n/a
Ovary carcinoma		4/4	n/a
Bladder carcinoma		42/42	36/36
- lymph node metastasis	1/1 strong		
- squamous carcinoma metastasis		2/2	
Lung - small cell carcinoma		1/1	5/5
- adenocarcinoma	5/5 strong		
Stomach adenocarcinoma		3/3	3/3
Liver carcinoma		4/4	4/4
Soft tissue tumors		3/3	n/a
Melanoma		48/48	18/18
- metastasis		18/18	

L-DOS47 Binds Primary and Metastatic human cancer tissues

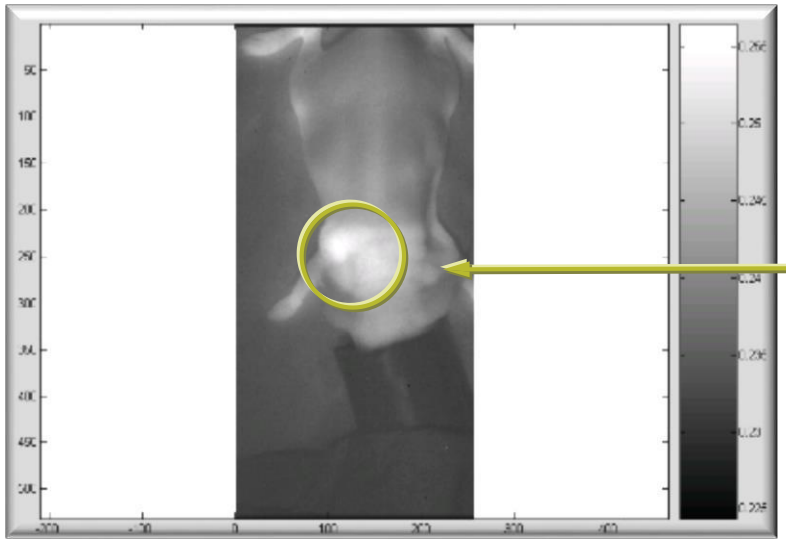


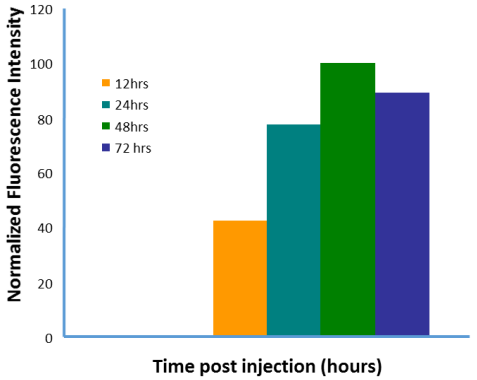
Human lung adenocarcinoma tissue biopsies were sectioned and prepared into slides. Positive binding of L-DOS47 is revealed by brown staining with blue counter stain.



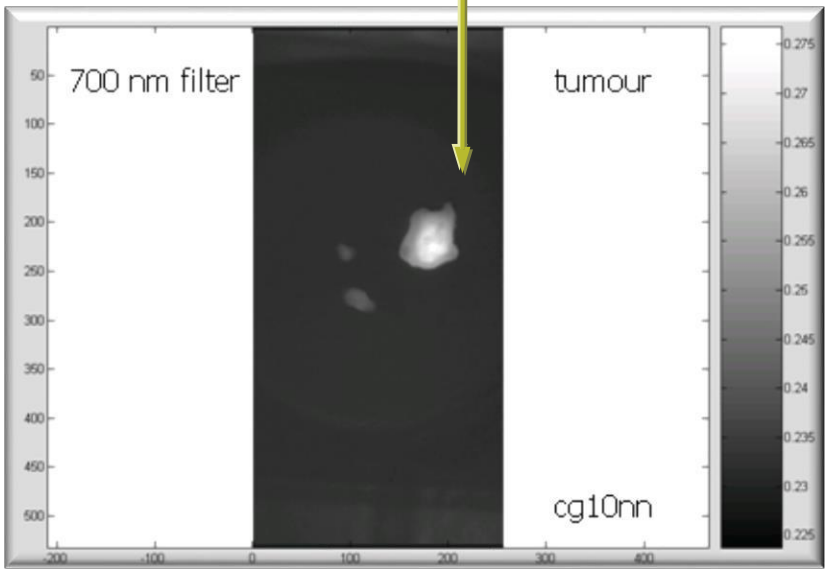
Immunopositive staining of L-DOS47 in human lung adenocarcinoma metastasized to lymph node. Positive binding of L-DOS47 is revealed by brown staining with blue counter stain

Specific Delivery to Tumors





Tumour specific localization



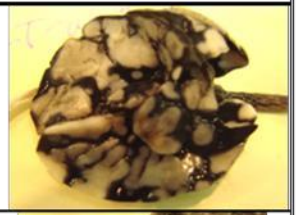
Full Body Scan

A549 tumour (8 x 7 mm)
L-DOS47-Cy5.5

Filtered Scan

L-DOS47-Cy5.5
Cy5.5 emission max
@710nm

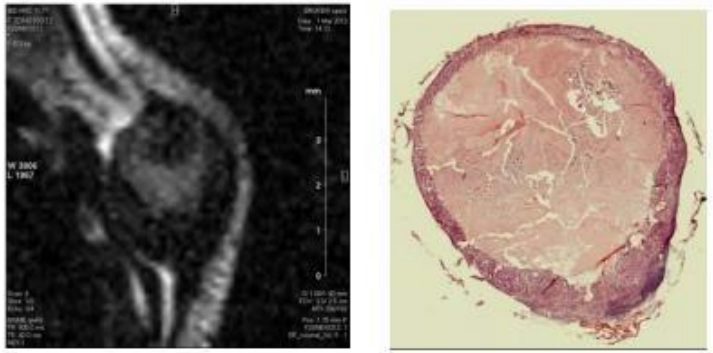
Tumour Formation Inhibition

Group	Cell Treatment	Final Concentration (µg/mL)	Mean number of lung tumors# 3 weeks	Mean number of lung tumors# 10 weeks	Representative lung images
1	Untreated	-	103.8 ± 30.0	110.6 ± 50.0	
2	Isotype	10	44.6 ± 5.1	60.4 ± 14.3	
3	L-DOS47	10	28.0* ± 7.2	50.0 ± 17.7	
4	L-DOS47	15	18.2* ± 7.8	112.2 ± 52.5	

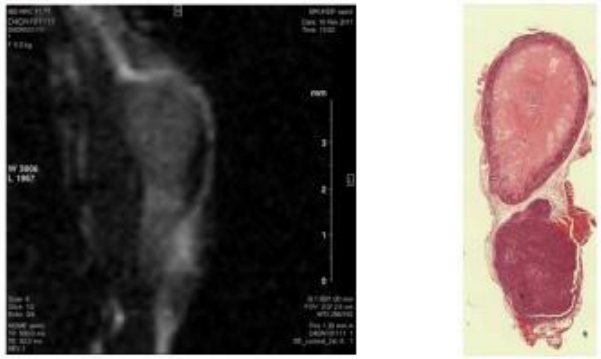
L-DOS47 Action Monitored by NMR

1H-NMR anatomical imaging

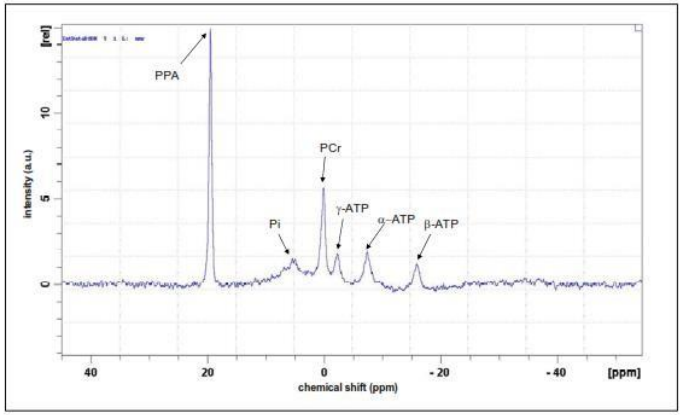
A control

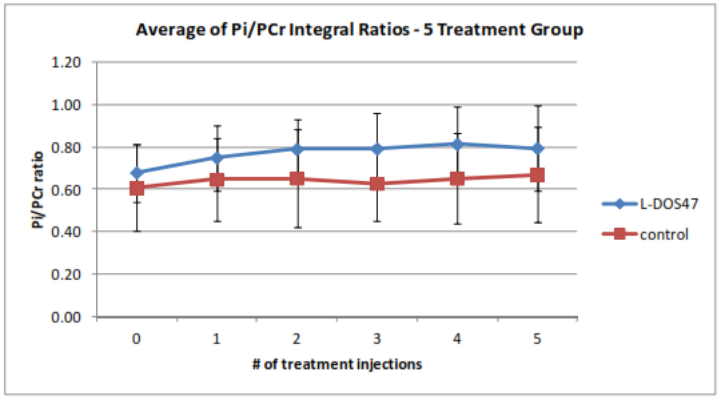


B Treatment



32P-NMR microenvironment





NMR imaging on A549 xenograft mice showing a change in energy metabolism (Pi/Pcr) as a result of L-DOS47 treatment

L-DOS47 Clinical Update

- L-DOS47 Phase I / II Trial (LDOS002)
  - Monotherapy in advanced NSCLC patients
  - Currently enrolling Phase II patients
- L-DOS47 Phase I with Expansion Trial (LDOS001)
  - Combination with pemetrexed and carboplatin
  - Currently enrolling in cohort 2
- L-DOS47 Phase II (LDOS003)
  - Combination with vinorelbine and cisplatin
  - In the planning phase

L-DOS47 Phase I / II Trial (LDOS002)

- Monotherapy treatment protocol in NSCLC patients that have not responded to other treatments;
- Stage IIIb / IV, metastatic, and progression after several lines of chemo, rad, surgery or chemo-naïve patients that have refused other lines of therapy;
- Dosed once a week for 2 weeks, 1 week rest (3-week cycle);
- Conducted in 5 Centers in Poland to assess safety (phase I) and then preliminary efficacy (phase II);
- Centers include The Maria Sklodowska-Curie Institute of Oncology, Military Institute of Health Institute, Mazovian Centre of Pulmonary Diseases and Tuberculosis in Otwock, Department of Oncology, Poznan University of Medical Sciences, National Tuberculosis and Lung Diseases Research Institute
- Phase II dosing regimen changed to twice a week dosing for 2 weeks, 1 week rest (3-week cycle);

Demography and NSCLC Baseline Characteristics (up to 12 Cohorts)

Demography	Total (N=40)	NSCLC History	Total (N=40)
Age	*Mean = 61.2* *Min, Max (34, 83)*	**Tumor Histology**	*Adeno = 38 (95%)* *Large Cell = 1 (2.5%)* *Unknown = 1 (2.5%)*
Weight (kg)	*Mean = 69.1* *Min, Max (48, 95)*	**Tumor Staging**	*Stage IIIB = 7 (17.5%)* *Stage IV = 33 (82.5%)*
Gender **Male** **Female**	*21 (52.5%)* *19 (47.5%)*	**Prior Therapy**	*None = 8 (20%)* *Chemo/Target = 32 (80%)* *Radiation = 21 (52.5%)* *Surgery = 11 (27.5%)*
Race **Caucasian**	*40 (100%)*	**Prior Chemo/Target Therapy**	*Adjuvant = 2 (5%)* *Locally Advanced = 3 (7.5%)* *Metastatic Disease = 31 (77.5%)* *None = 9 (22.5%)*
ECOG **0** **1** **2**	*11 (27.5%)* *27 (67.5%)* *2 (5%)*	**Best Response**	*Unknown = 5 (16.1%)* *CR = 1 (3.2%)* *PR = 9 (29%)* *Stable = 9 (29%)* *PD = 7 (22.6%)*

Clinical Observations Up to Cohort 12

- 21/40 patients had an overall response of SD at cycle 2;
- 10/40 patients had an overall response of SD at cycle 4;
- Patient 01-047 enrolled in cohort 9 (1.84µg/kg) was progression free for 10 cycles (approx. 7 months);
- None of the patients treated to-date have had a partial or complete response as defined by RECIST v1.1 definition;
- One DLT reported in cohort 13 (Grade 4 Back Pain);
- Trial Steering Committee approved enrollment of patients to Phase II at the cohort 16 dose (13.55µg/kg).

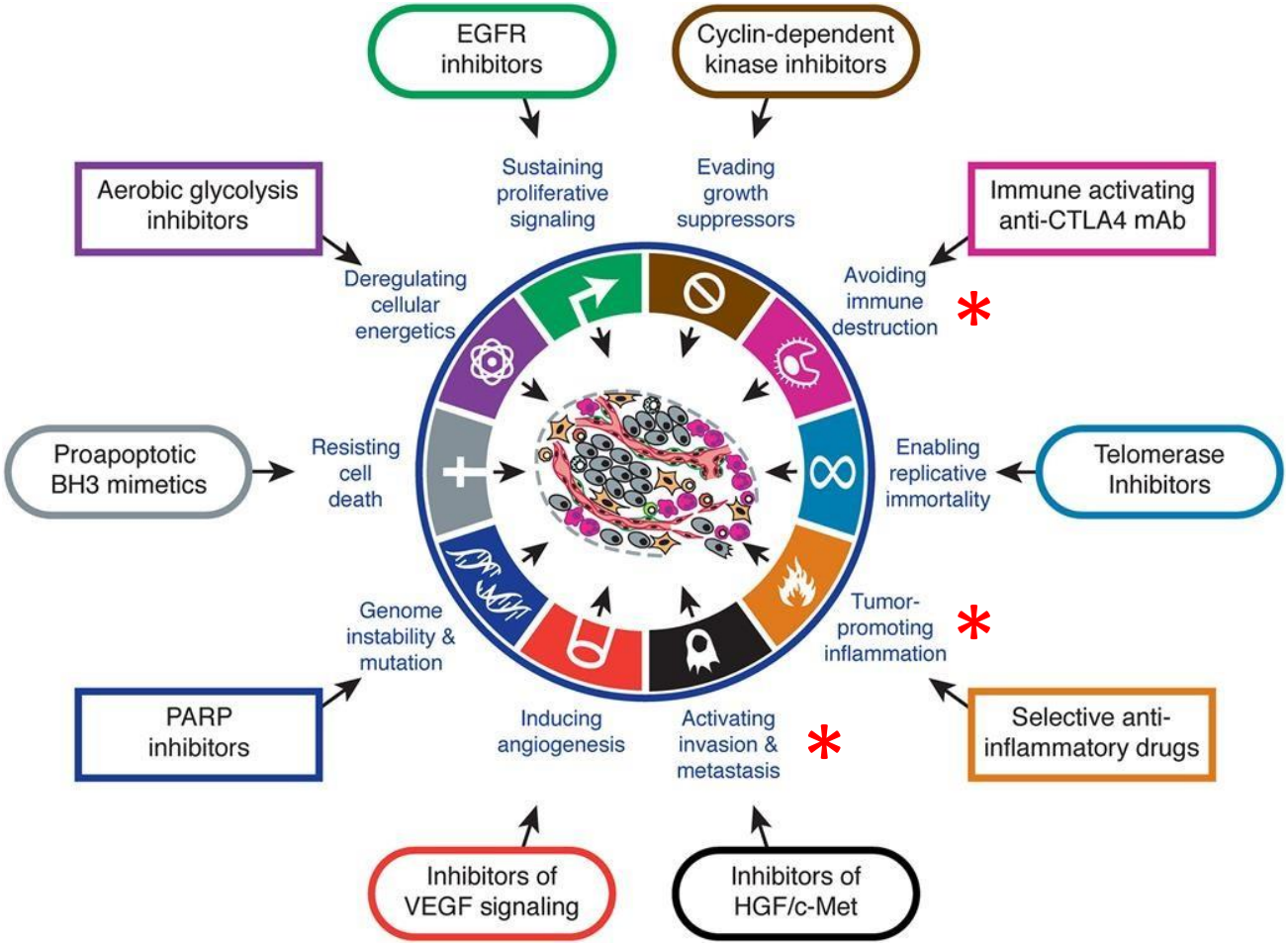
L-DOS002 Summary of Results

- One Dose Limiting Toxicity (DLT);
- No safety issues beyond those observed in pre-clinical toxicology studies or expected in the population of patients being studied;
- Immunogenicity consistent with what was observed pre-clinically;
- Clinical trends observed to-date are encouraging;
- Phase II currently enrolling.

L-DOS47 Phase I with Expansion Trial (LDOS001)

- Combination therapy in first-line treatment of NSCLC:
  - Stage IIIb / IV, metastatic, and chemo-naïve;
  - Given in combination with standard pemetrexed/carboplatin treatment;
  - Dosed continuously each week;
  - Monitor radiologically every 6 weeks;
- Conducted in 3 Centers in US to assess safety (phase I) and then preliminary efficacy (expansion);
- Clinical sites include MD Anderson, Hershey Penn State, and Case Western;
- Two cohorts of patients enrolled to-date.

Tumor pH Treatment Strategy: DOS47*



Cancer Cell 2008, 13 472-482

Helix BioPharma Corp

THANK YOU