CO016

Oral Session APL Biology -II

Metabolic catastrophe of arsenic trioxide resistant cells in acute promyelocytic leukemia

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26th September 2017

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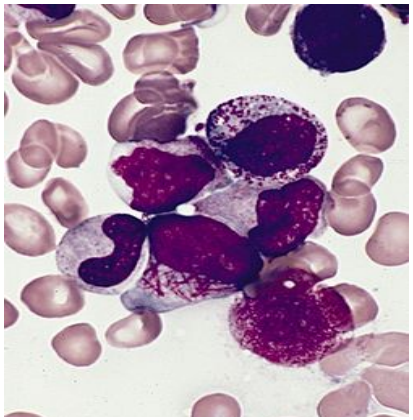
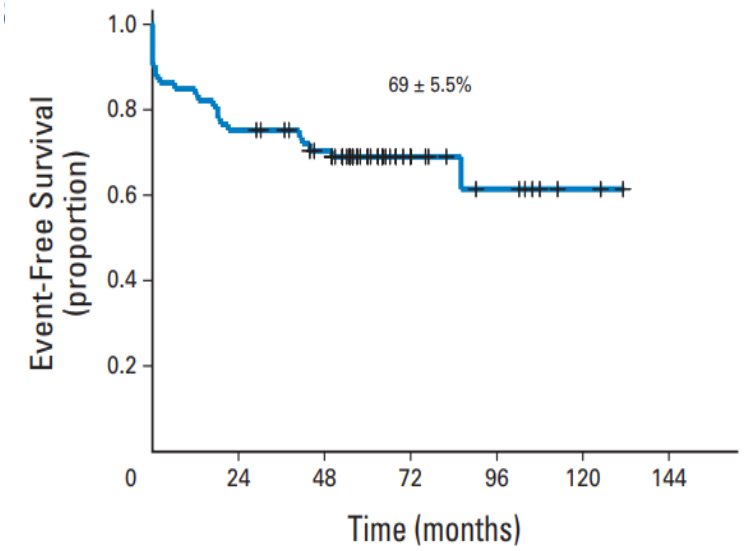
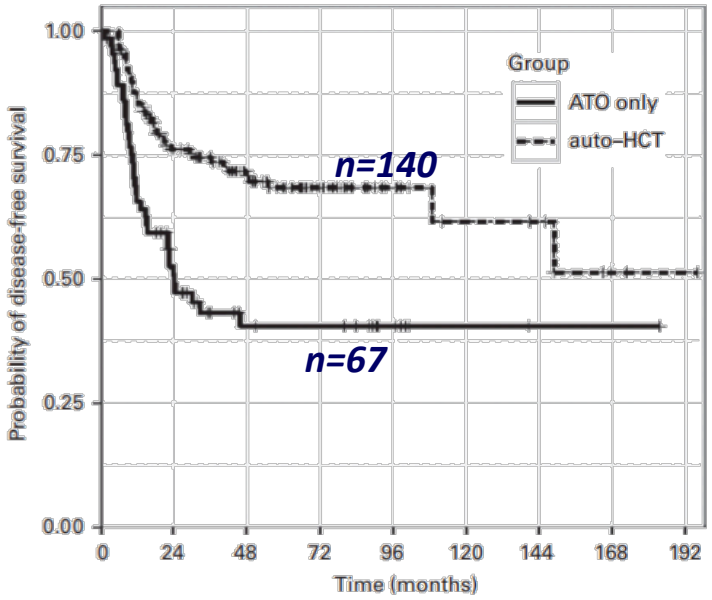
Acute Promyelocytic Leukemia



- >95% of APL cases – PML-RARA Fusion
- Combination of ATRA and ATO in patients with low-risk disease is very promising.
- Relapses with arsenic trioxide [1,2]:
  - Newly diagnosed APL (NAPL) Relapses 10-20%
  - Relapsed APL (RAPL) -Relapses 60%.

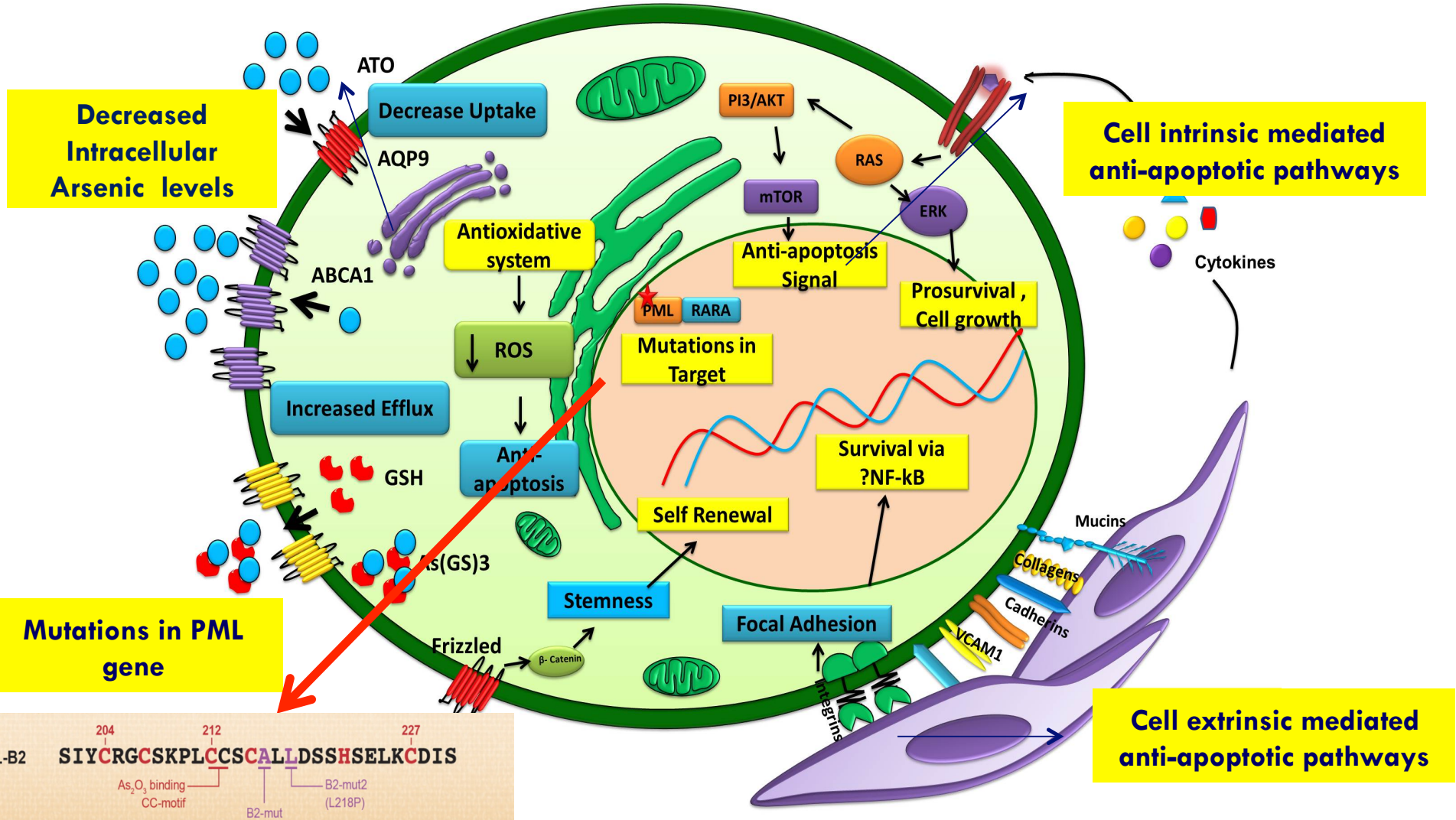




Limited data to explain this inferior long term clinical response in RAPL treated with ATO in contrast to NAPL

1Mathews V et al. Journal of Clinical Oncology (2010) Aug 28(24):3866-71;2 C Ganzel et al., Bone Marrow Transplantation (2016)

Potential Mechanisms of Resistance to ATO



Go to .et al.,Blood 2011;118(6):1600-1609

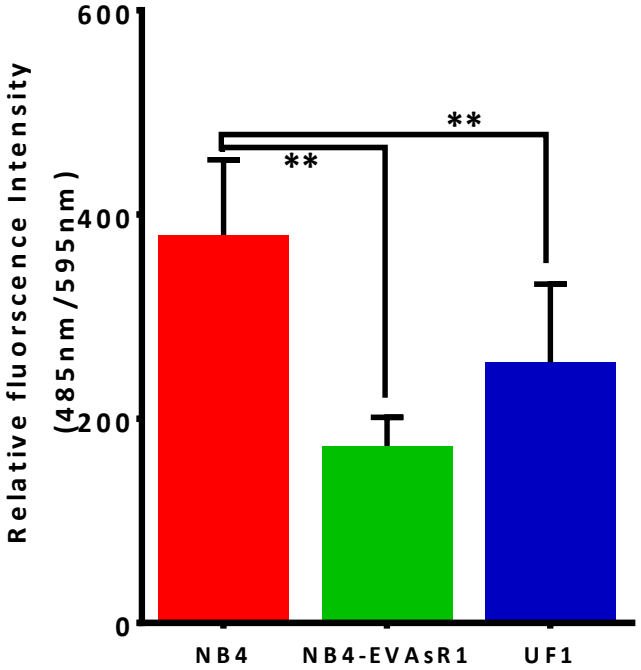
Arsenic trioxide resistance: More to it than mutations in PML-RARA

Characteristic features	*NB4 naive*	*NB4 EV-AsR1*	*NB4 EV-AsR2*	*NB4 EV-AsR3*	*UF1*
Presence of PML B2 domain mutation (A216V)	-	+ 91.7 % VAF	-	-	-
Sensitivity to ATO (IC 50 -µM)	0.9	3.09	3.44	2.88	4.1
Sensitivity to other chemotherapy a) Daunorubicin IC50(µM) b) Cytosine arabinoside IC50(µM)	 0.14 8.3	 0.22 16.5	 0.19 4.7	 0.2 13.1	 0.18 0.309
Reactive oxygen species (ROS) levels (MFI Fold difference normalized to NB4 cells) (n=3)	1	0.74	0.86	0.68	0.3
Glutathione levels (MFI Fold difference- normalized to NB4 cells) (n=3)	1	1.37	1.45	1.39	0.5
Doubling time (hrs)	**28**	**46**	**48**	**42**	**72**

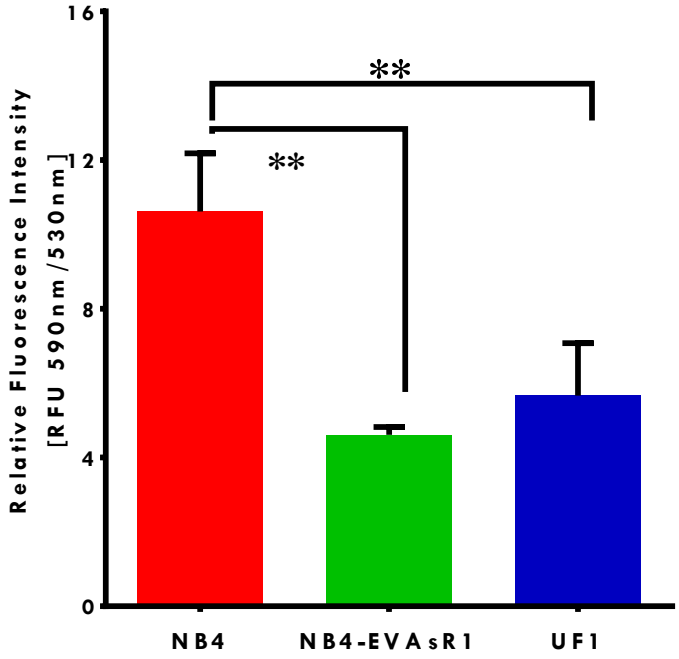
ASH 2014

Metabolic features of ATO resistant cell line

2-NBDG –Glucose Uptake



Mitochondrial Membrane Potential (MMP)

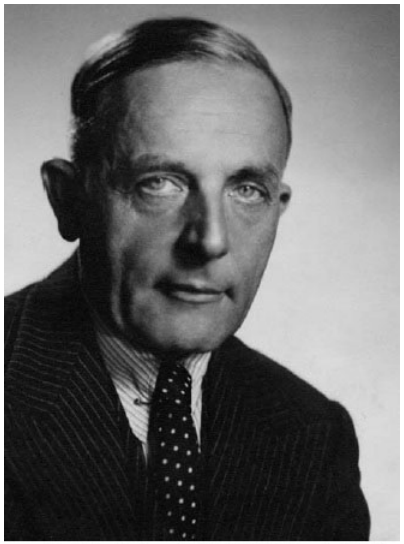


Sensitive Cell line : NB4,
Resistant cell line :
NB4-EvAsR1 and UF1
MMP (JC-1)
Glucose Uptake - 2-NBDG – analog of Glucose (n=3)
(n=4)

- **Lower glucose uptake, ROS, Mitochondrial Membrane potential and increased antioxidant level, doubling time in contrast to NB4 naive cell line suggests that the ATO resistant cells are metabolically less active.**

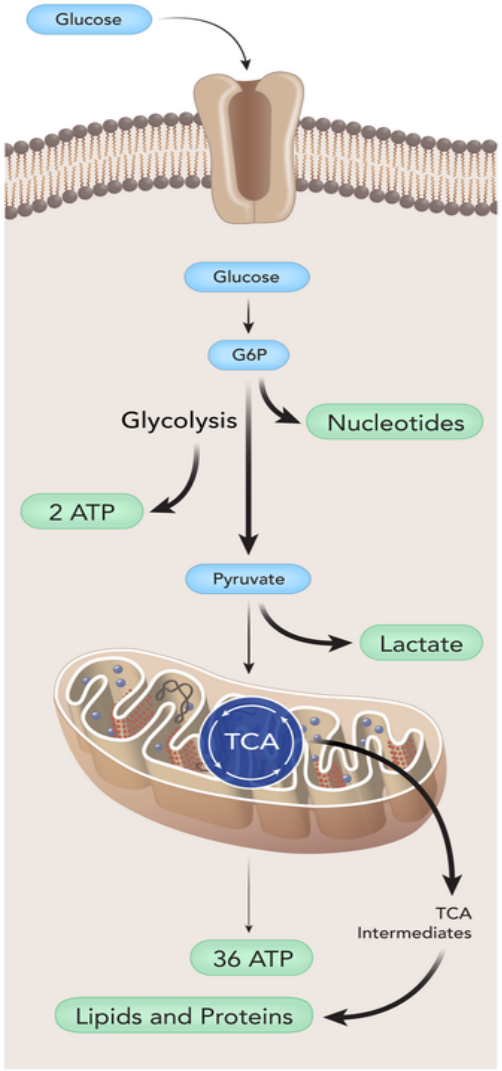
Metabolism of cancer cells are distinct from normal cells

"the rate of glucose uptake dramatically increases and lactate is produced, even in the presence of oxygen" – Aerobic Glycolysis – Warburg Effect



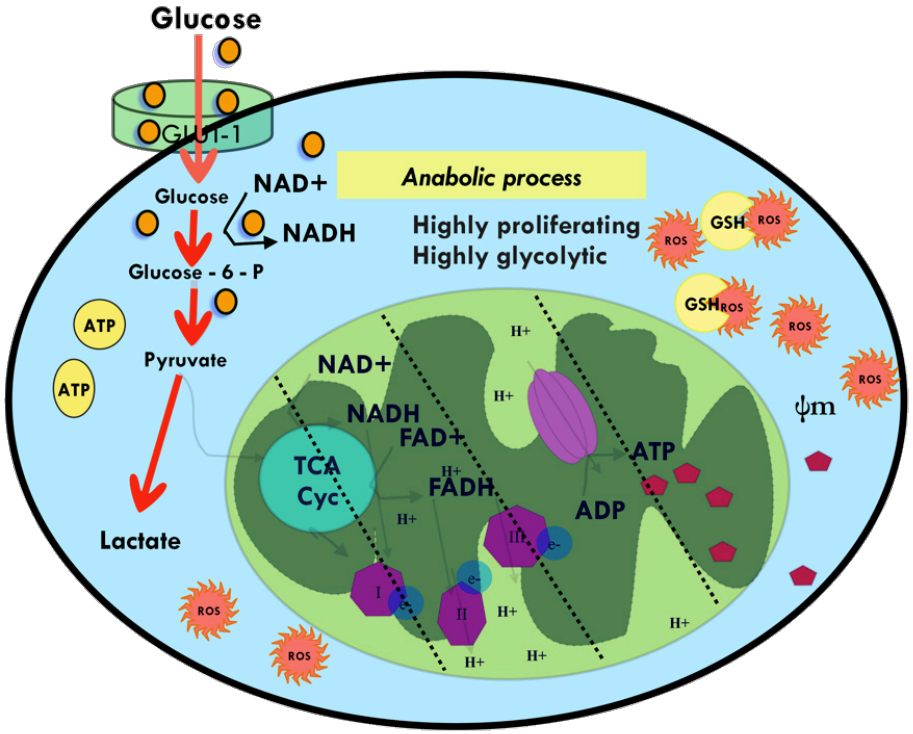
Dr. Otto Warburg (Oct 8, 1883 Aug 1, 1970)

Cell 144, March 4, 2011 ª2011 Elsevier Inc.

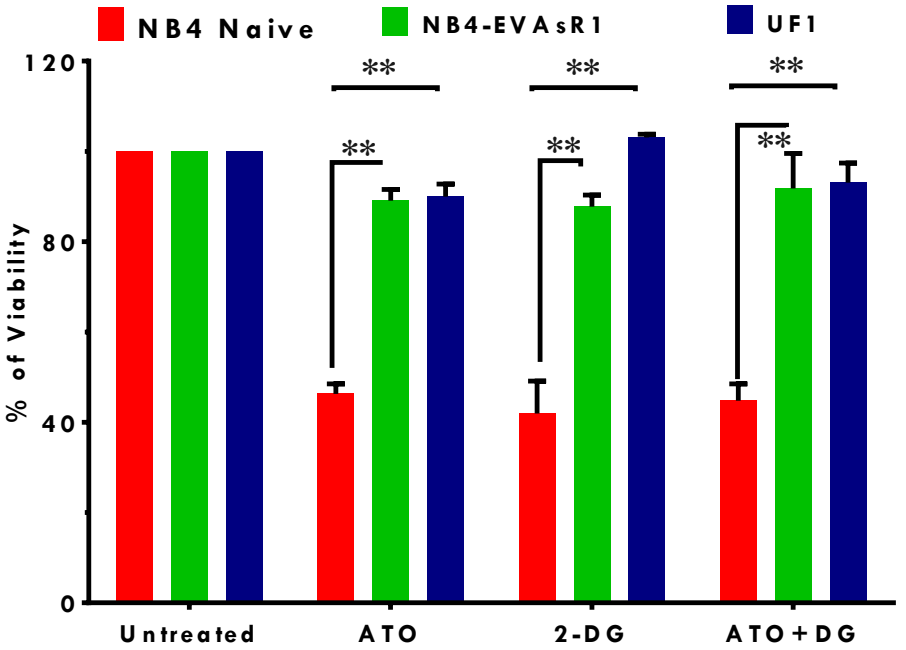


Cancer cells rely on glycolysis

Sensitivity to metabolic inhibitor – Glycolytic inhibition



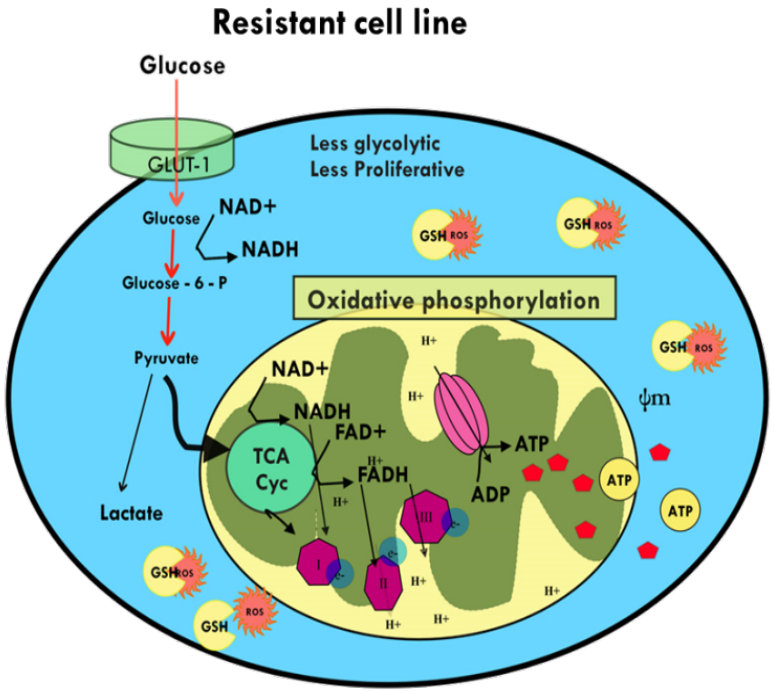
2-Deoxy Glucose (2-DG) – Inhibitor of Glycolysis

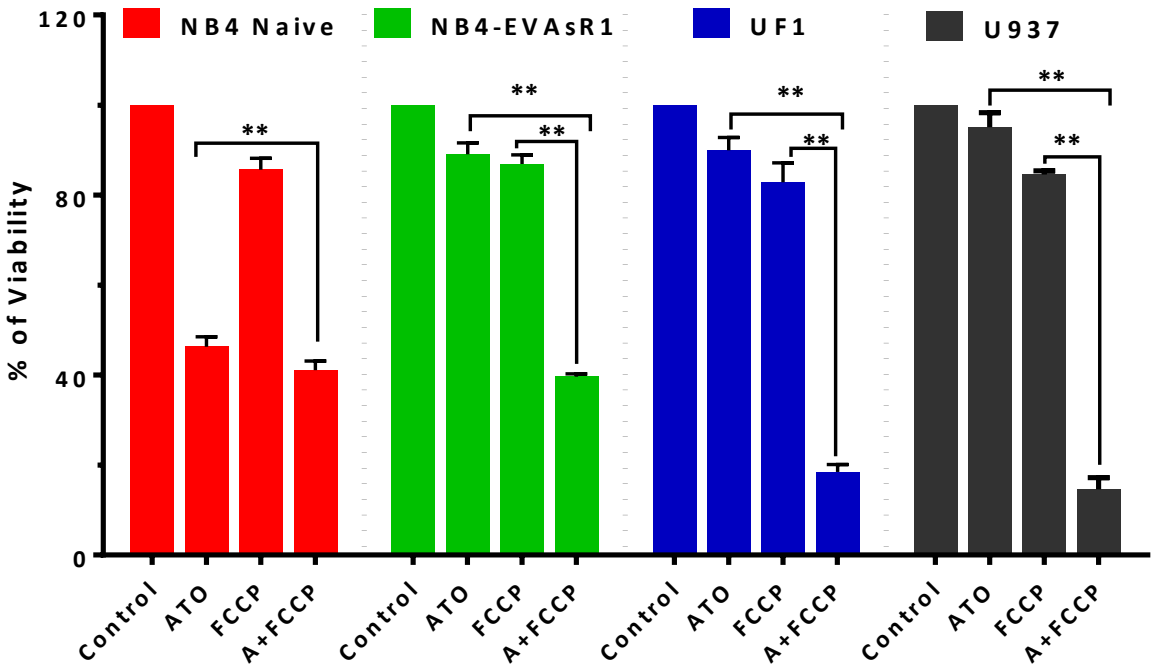


Cell line : NB4, NB4-EvAsR1 and UF1
Duration : 48hours (n=4)
Drugs : ATO (2uM): 2-DG (5mM)
Assay : Annexin V-7AAD

ATO resistant cell lines are not relying on glycolytic pathway for their survival and proliferation

Sensitivity to metabolic inhibitor – OXPHOS Inhibition

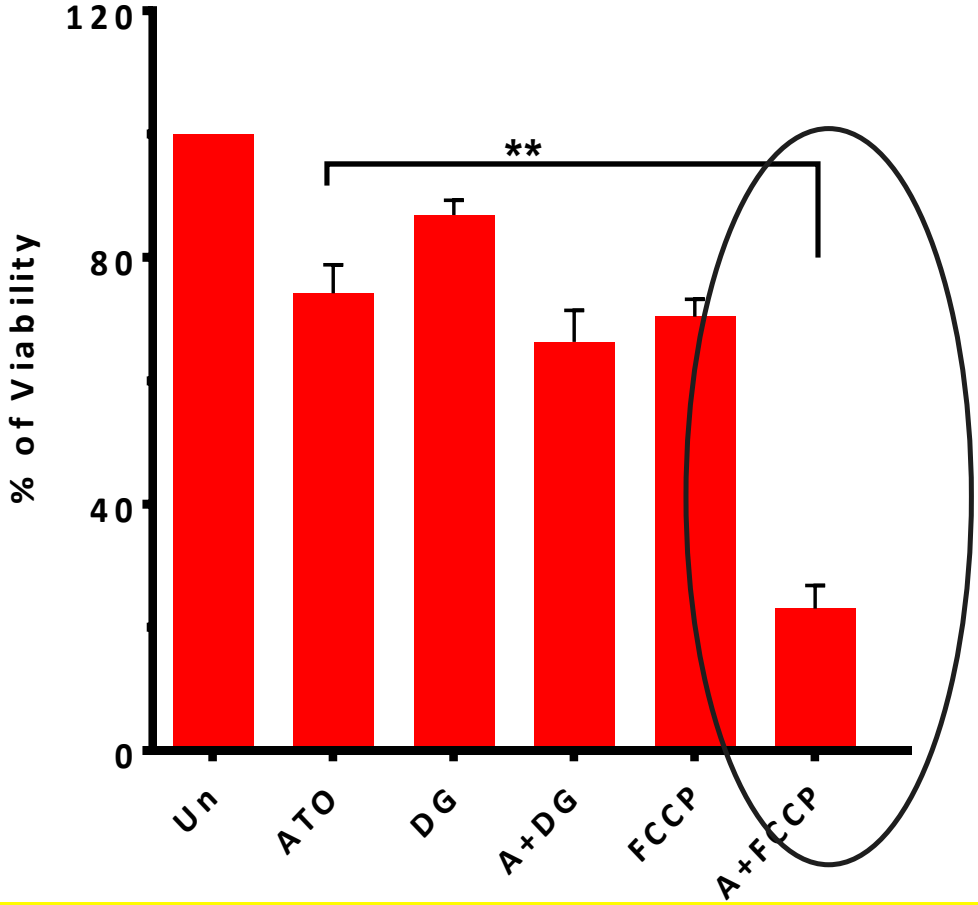




- FCCP – dissipates the membrane potential which is required for the activation of ATP synthase complex to generates ATP.

Cell line : NB4, NB4-EvAsR1 ,UF1 and U937
Duration : 48hours; (n=4)
Drugs : ATO (2uM);2-DG (5mM); FCCP (10uM)
Assay : Annexin V-7AAD

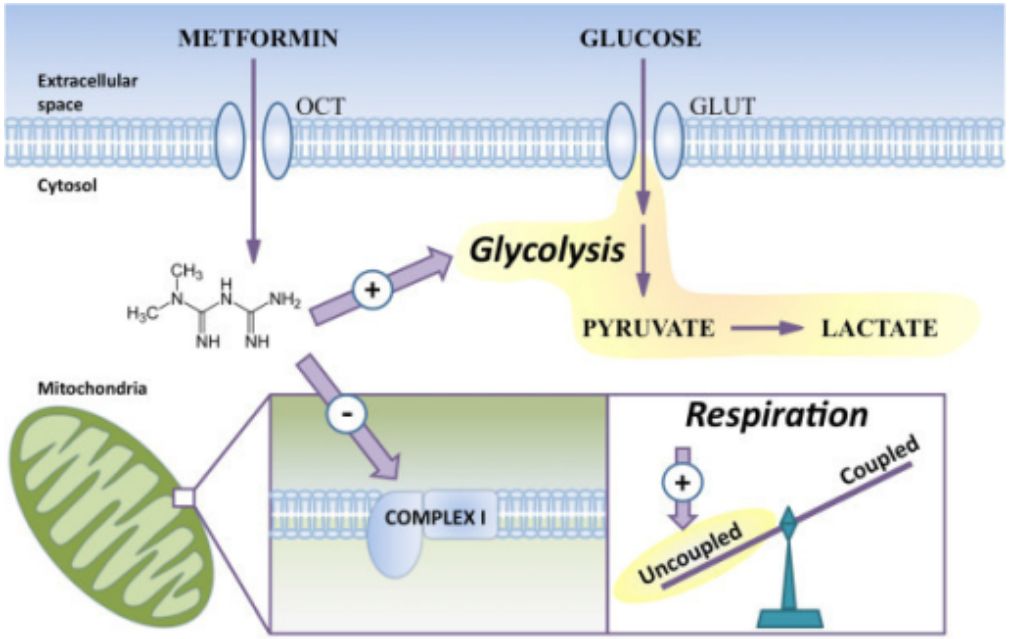
Effect of metabolic disruptors on normal cells



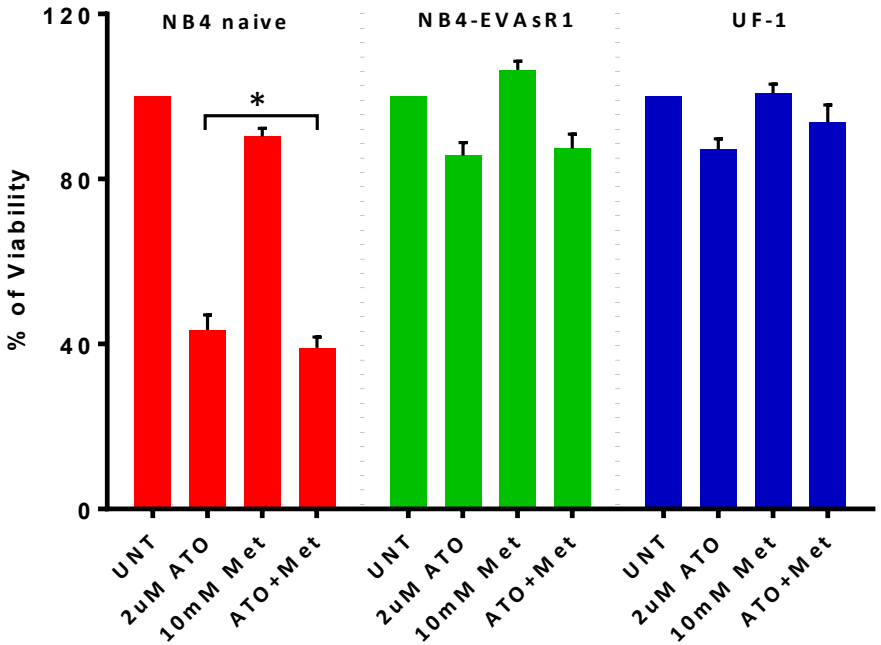
Normal Peripheral blood mononuclear cell
Duration : 48hours
Drugs : ATO (2uM);2-DG (5mM); FCCP (10uM)
Assay : Annexin V-7AAD
(n=4)

Despite of the profound effect of ATO+FCCP on malignant cells there is a significant bystander effect on the normal peripheral blood mononuclear cell which limits it potential to be translated into the clinic

Effect of Metformin (OXPHOS Complex –I inhibitor) on ATO resistant cell lines



Andrzejewski et al. Cancer & Metabolism 2014,



Inhibition of Complex-I is not effective in overcoming resistance to ATO, the cells might survive using other energy sources of TCA cycle intermediates (glutamine, a- keto glutarate) to drive OXPHOS surpassing complex I inhibition or by up regulating glycolysis

www.aging-us.com AGING 2016, Vol. 8, No. 8

Research Paper

Bedaquiline, an FDA-approved antibiotic, inhibits mitochondrial function and potently blocks the proliferative expansion of stem-like cancer cells (CSCs)

Marco Fiorillo[1,2,3], Rebecca Lamb[1,2], Herbert B. Tanowitz[4], Anna Rita Cappello[3], Ubaldo E. Martinez-Outschoorn[5], Federica Sotgia[6], and Michael P. Lisanti[1,2]

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[2]The Manchester Centre for Cellular Metabolism (MCCM), Institute of Cancer Sciences, Cancer Research UK Manchester Institute, University of Manchester, Manchester, UK
[3]The Department of Pharmacy, Health and Nutritional Sciences, The University of Calabria, Cosenza, Italy
[4]Departments of Pathology and Medicine, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[5]The Sidney Kimmel Cancer Center, Philadelphia, PA 19107, USA
[6]School of Environment & Life Sciences, University of Salford, Salford, UK

Bedaquiline is an FDA-approved anti-microbial drug, selectively sensitizes the malignant cells by targeting the mitochondrial ATP-synthase , leading to mitochondrial dysfunction and ATP depletion.

Summary

ATO Sensitive cell line

- High ψm
- Increased ROS
- Decreased Glutathione
- Increased Proliferation rate

ATO Resistant cell line

- Low ψm
- Decreased ROS
- Increased Glutathione
- Decreased Proliferation rate

- Resistance to ATO is not restricted to mutations in PML-RARA and that it is likely to be multi-factorial.
- ATO resistant cell lines survives the energy crisis by efficiently handling the two metabolic pathways (Glycolysis and OXPHOS) when one is inhibited.
- There are a number of FDA approved molecules widely used in the clinic and are reported to have inhibitory effect on malignant cells mitochondrial respiration.
- Targeting the metabolic adaptation could be a potential approach to overcome arsenic trioxide resistance.

Acknowledgements

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Christian Medical College, Vellore,India

Faculty:

Dr. Vikram Mathews

Dr. Alok Srivastava

Dr. Biju George

Dr. Poonkuzhali Balasubramanian

Dr.Uday Kulkarni

Lab members:

Ezhilarasi Chendamarai

Saravanan Ganesan

Hamenth Kumar Palani

Ansu Abu Alex

Sachin David

Arvind Venkatraman

Funding:

Dr. Vikram Mathews – Senior fellowship from Wellcome Trust/ DBT.

Thank you