33rd AICC ANNUAL CONFERENCE: INTERNATIONAL MEETING ON CANCER METABOLISM
22-24th November 2021
Turin, Italy

Primary bone marrow-derived mesenchymal stem cells secrete citrate and take up glutamate under physiological culture conditions

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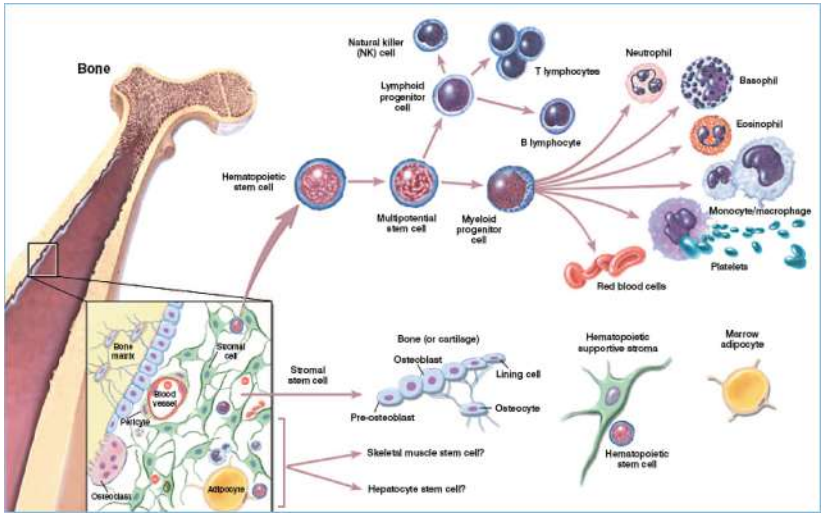
University of Parma

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent cells of the bone marrow niche, able to:

- ❑ differentiate into osteoblasts, chondroblasts, and adipocytes
- ❑ migrate towards the sites of injury
- ❑ modulate immune response
- ❑ support haematopoiesis



http://stemcells.nih.gov/info/2006report/2006chapter2.htm

In neoplastic bone marrow, MSCs arrange metabolic exchanges with cancer cells that can:

- ❑ alter MSC properties (differentiation, immunoregulation…)
- ❑ promote survival and enhance drug resistance of cancer cells

frontiers in Cell and Developmental Biology

The Role of Amino Acids in the Crosstalk Between Mesenchymal Stromal Cells and Neoplastic Cells in the Hematopoietic Niche

Martina Chiu, Giuseppe Taurino, Massimiliano G. Bianchi and Ovidio Bussolati**

Department of Medicine and Surgery, University of Parma, Parma, Italy

MSCs in neoplastic bone marrow niches

cancers

MDPI

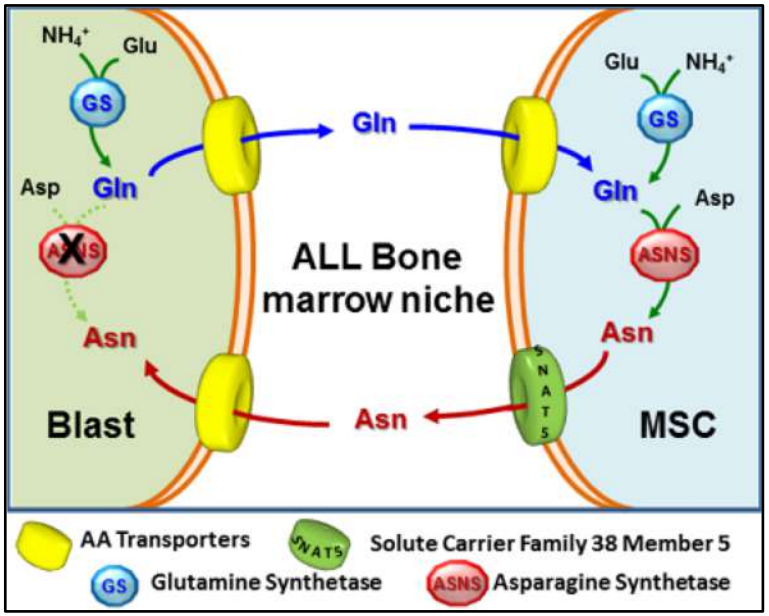
Article

Myeloma Cells Deplete Bone Marrow Glutamine and Inhibit Osteoblast Differentiation Limiting Asparagine Availability

Martina Chiu [1,†], Denise Toscani [1,†], Valentina Marchica [1], Giuseppe Taurino [1], Federica Costa [1], Massimiliano G. Bianchi [1], Roberta Andreoli [1], Valentina Franceschi [2], Paola Storti [1], Jessica Burroughs-Garcia [1,2], Rosa Alba Eufemiese [1], Benedetta Dalla Palma [1,3], Nicoletta Campanini [4], Eugenia Martella [4], Cristina Mancini [4], Jixiu Shan [5], Michael S. Kilberg [5], Giovanna D'Amico [6], Erica Dander [6], Luca Agnelli [7], Giancarlo Pruneri [7], Gaetano Donofrio [2], Ovidio Bussolati [1,*] and Nicola Giuliani [1,3,*]

Chiu M., Toscani D., ***Myeloma cells deplete bone marrow glutamine and inhibit osteoblast differentiation limiting asparagine availability.*** *Cancers. 2020*

In **Multiple Myeloma** bone marrow niche, glutamine depletion by glutamine-addicted malignant plasma cells hinders MSC differentiation into osteoblasts → **Osteolytic lesions**



Chiu M., Taurino G., Dander E., ***ALL blasts drive primary mesenchymal stroma cells to increase asparagine availability during Asparaginase treatment.*** *Blood Advances. 2021*

In **Acute Lymphoblastic Leukemia** bone marrow niche, Asn-auxotroph blasts synthesize and secrete Gln, that, in turn, is used by MSCs to produce Asn, which is then exported through the transporter SNAT5 (induced in MSCs by ALL blasts) and provided to leukemic cells

The amino acid trade-off depends on Glutamine Synthetase (GS) and the transporter SNAT5

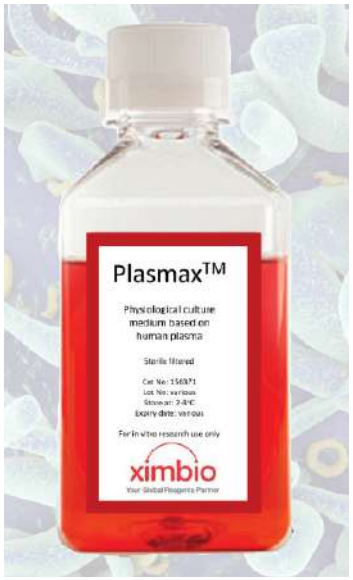
ALL blasts survival during L-Asparaginase treatment

Increasing evidence for an important metabolic role for MSCs in neoplastic bone marrow, but their metabolic profile is not yet chracterized

Aim of the study

We have profiled the metabolism of primary bone marrow mesenchymal stem cells derived from different donors (n=8, provided by A. Biondi and G. D'Amico, Centro di Ricerca M. Tettamanti, University of Milano-Bicocca, S. Gerardo Hospital, Monza) **under physiological conditions:**

- ❑ under «hypoxic» conditions (1%O_2) in a Whitley H35 Hypoxystation (Don Whitley Scientific)
- ❑ in a plasma-like advanced medium (Plasmax™)



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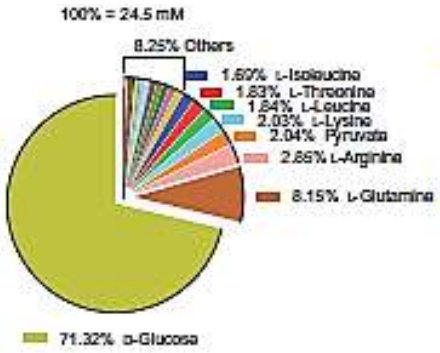
Plasmax™ cell culture medium

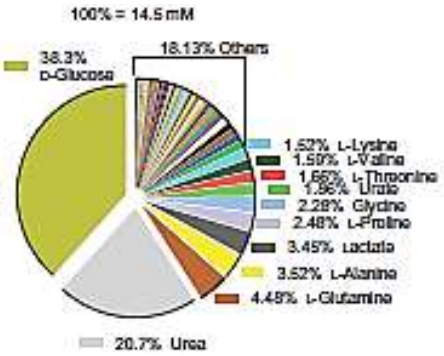
PATENTED PATENTED PATENTED

	Plasmax™	MEM	IMDM	DMEM	DMEM/ F-12	F-12	RPMI 1640
Proteinogenic Amino Acids							
L-Alanine	510		281		50	100	
L-Arginine	64	597	399	398	699	1000	1149
L-Asparagine	41		189		50	100	379
L-Aspartic acid	6		226		50	100	150
L-Cysteine	33				100	200	
L-Glutamate	98		510		50	100	136
L-Glutamine	650	2000	4000	4000	2500	1000	2055
Glycine	330		400	400	250	100	133
L-Histidine	120	200	200	200	150	100	97
L-Isoleucine	140	397	802	802	416	31	382
L-Leucine	170	397	802	802	451	100	382
L-Lysine	220	399	798	798	499	199	219
L-Methionine	30	101	201	201	116	30	101
L-Phenylalanine	68	194	400	400	215	30	91
L-Proline	360		348		150	300	174
L-Serine	140		400	400	250	100	286
L-Threonine	240	403	798	798	449	100	168
L-Tryptophan	78	49	78	78	44	10	25
L-Tyrosine	74	199	462	399	214	30	111
L-Valine	230	393	803	803	452	100	171
Other Components							
Acetate	42						
Acetone	55						
Acetyl carnitine	5						
Citrate	114						
Carnitine	46						
Creatine	37						
Creatinine	74						
Formate	33						
D-Glucose	5556	5556	25000	25000	17506	10011	11111
Glycerol	82						
2-Hydroxybutyrate	31						
3-Hydroxybutyrate	77						
3-Hydroxyisobutyrate	20						
Hypoxanthine	5				15	30	
Lactate	500						

DMEM F-12



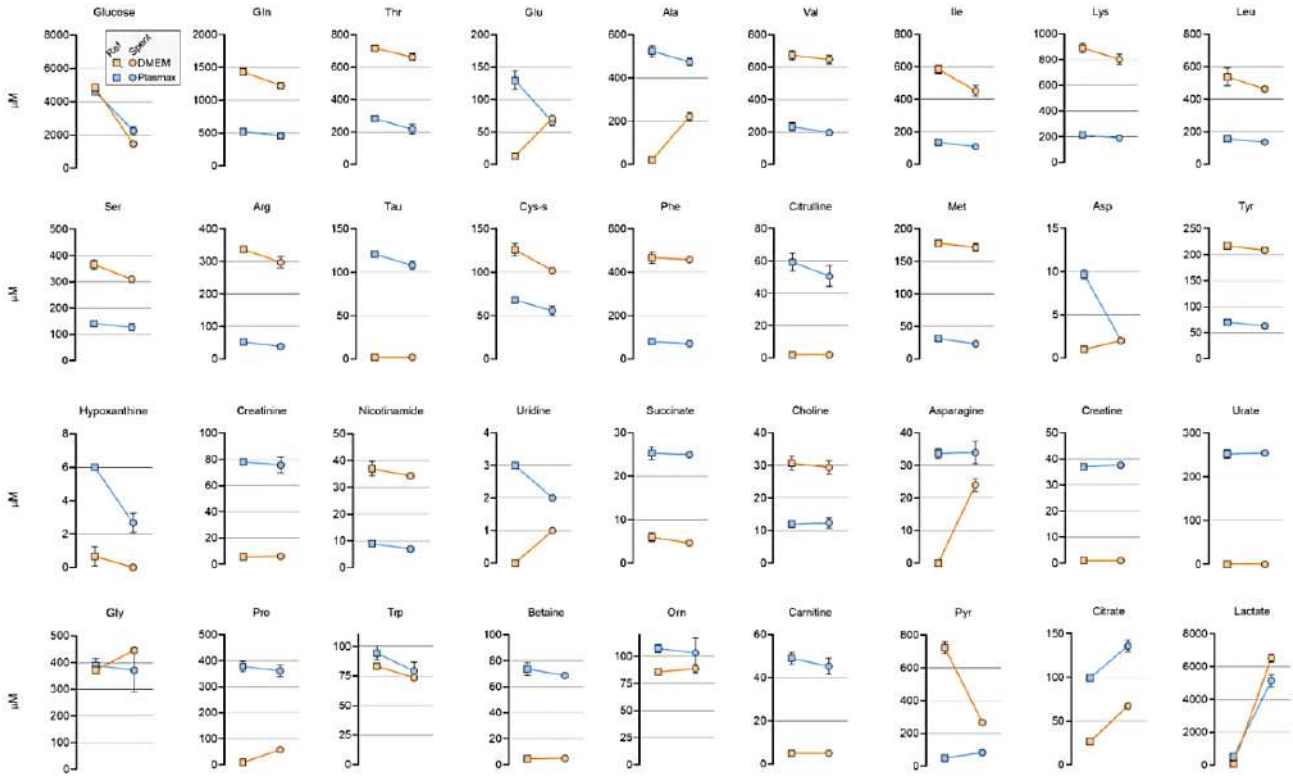
PLASMAX™

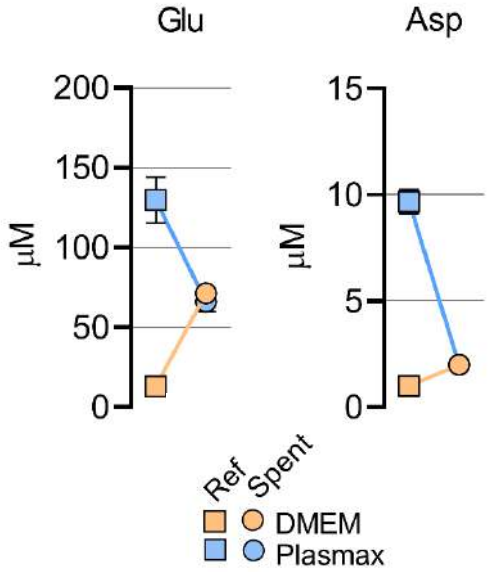


Comparison of the formulation of Plasmax™ and DMEM (Van Voorde et al. 2019)

Plasmax is an advanced cell culture medium which contains more than 80 components and mimics the metabolic profile of normal human plasma

Plasma-like medium *vs.* standard culture medium



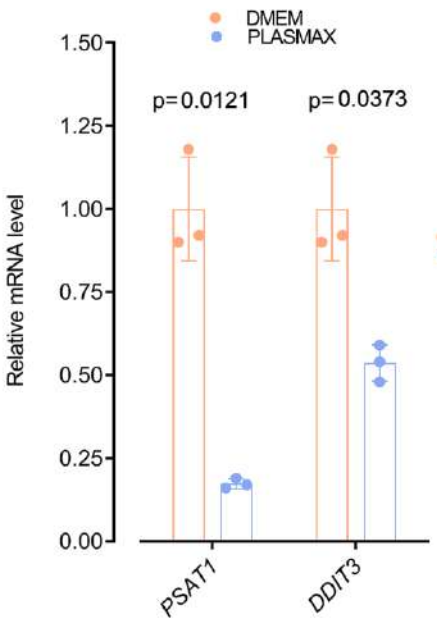


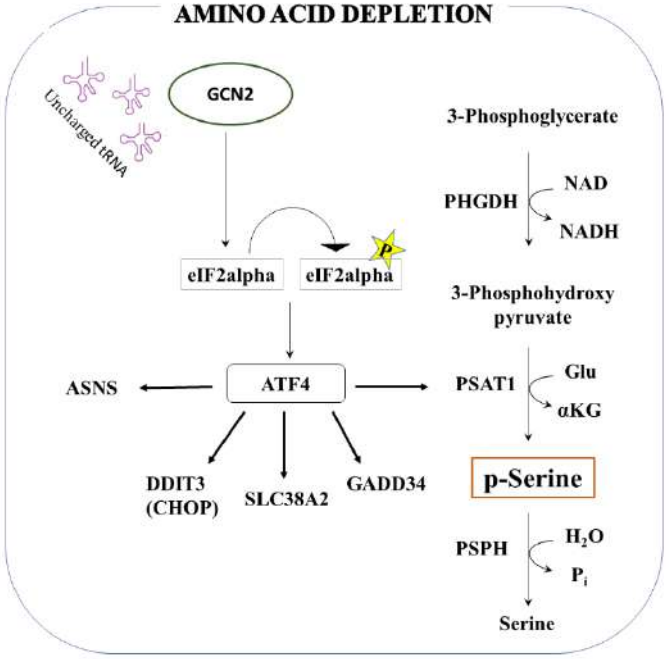
*In standard growth medium (**DMEM**) exchange rates of MSCs are significantly different from those determined in **Plasmax™***

When cultured in Plasmax™ MSC consume high levels of Glu and Asp, suggesting a metabolic role for these anionic amino acids

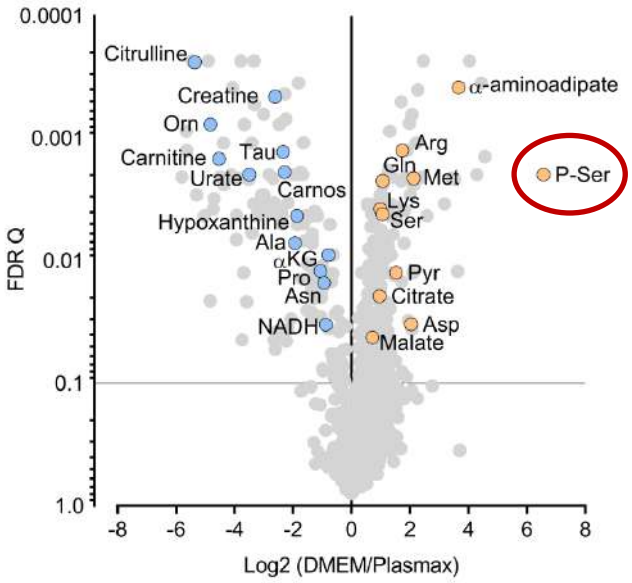
Plasma-like medium *vs.* standard culture medium

Nutritional stress markers



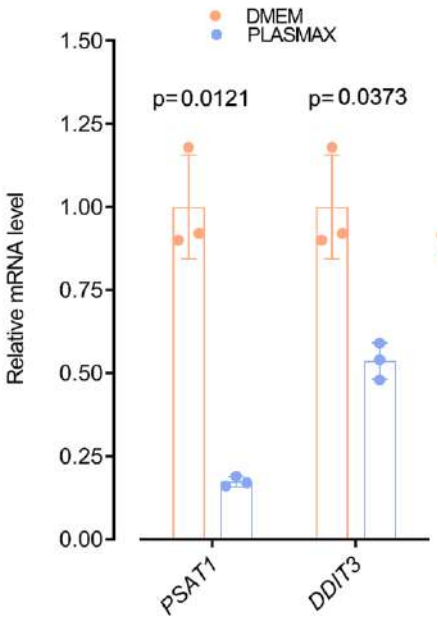


Intracellular metabolites (untargeted analysis)

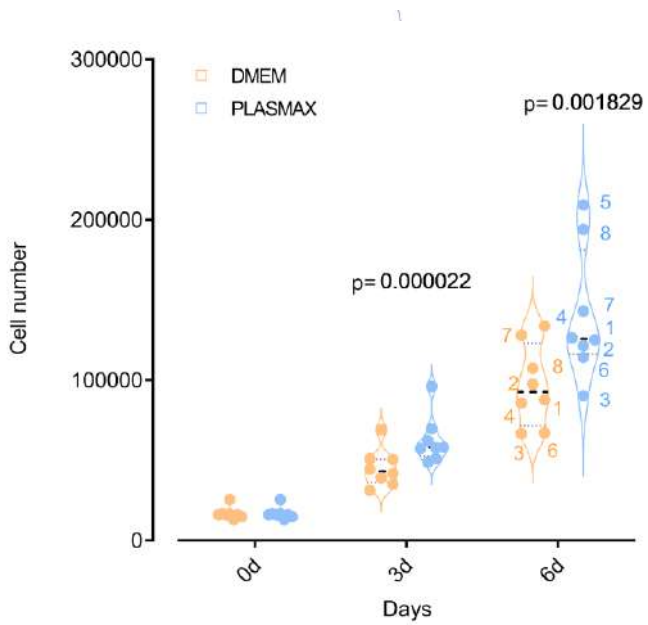


Plasma-like medium *vs.* standard culture medium

Nutritional stress markers



Cell proliferation

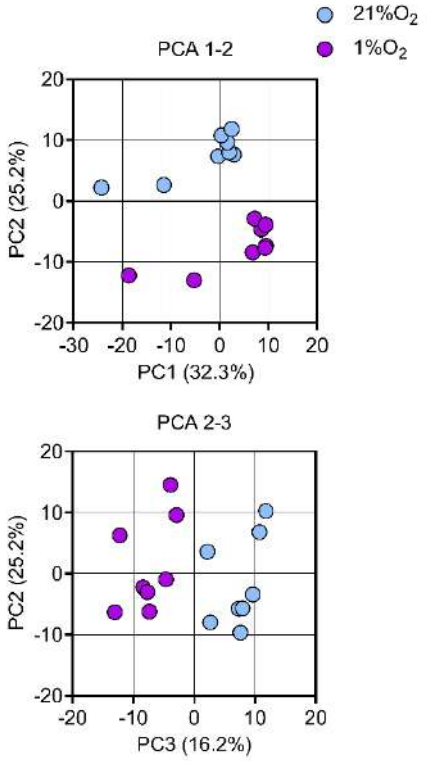


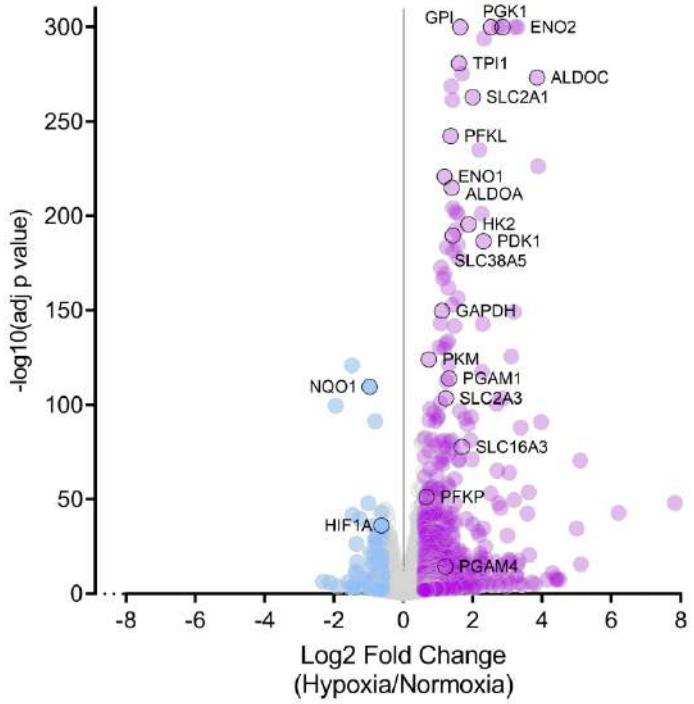
In DMEM MSCs undergo a severe nutritional stress and grow less than in Plasmax™

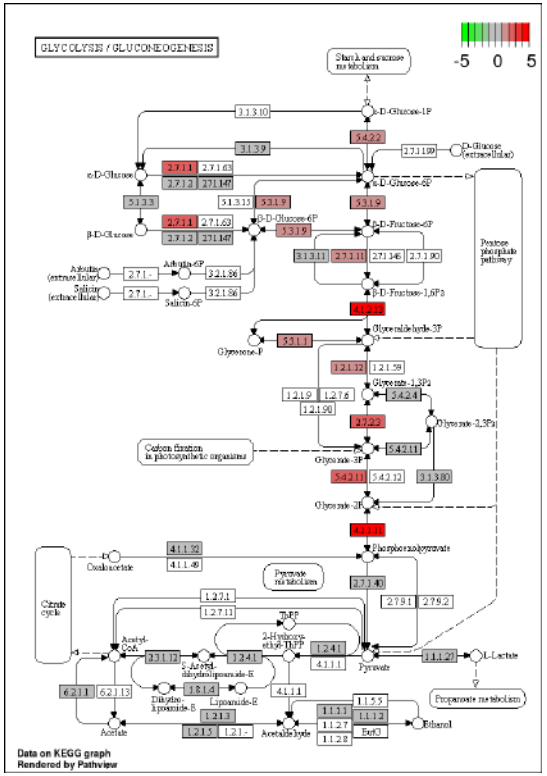
Standard cell culture media severely alter the physiological metabolic profile and proliferation rate of MSCs

Gene expression: normoxia *vs.* hypoxia

***"Normoxia"**: 21%O_2 **"Hypoxia"**: 1%O_2, closer to physiological bone marrow conditions*

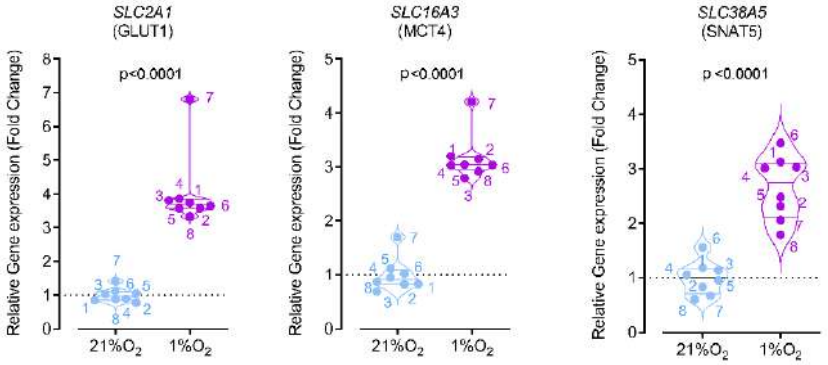




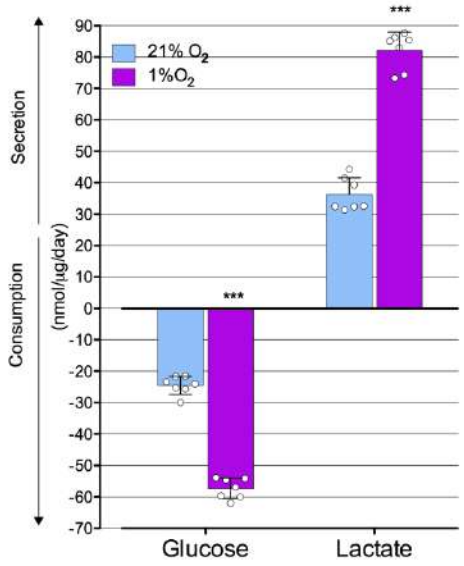


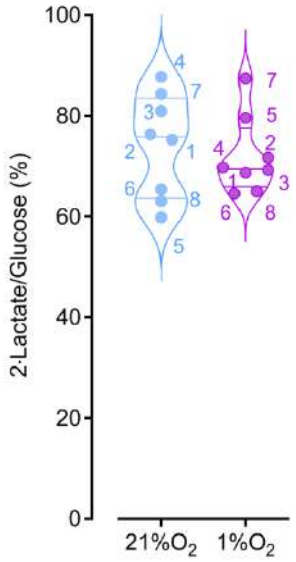
623 genes are upregulated under hypoxic conditions:

1) **glycolitic enzymes, PDK1**
2) **glucose transporters *SLC2A1/3* (GLUT1/3)**
3) **the lactate transporter *SLC16A3* (MCT4)**
4) **the glutamine transporter *SLC38A5* (SNAT5)**



Glucose metabolism: glucose/lactate ratio

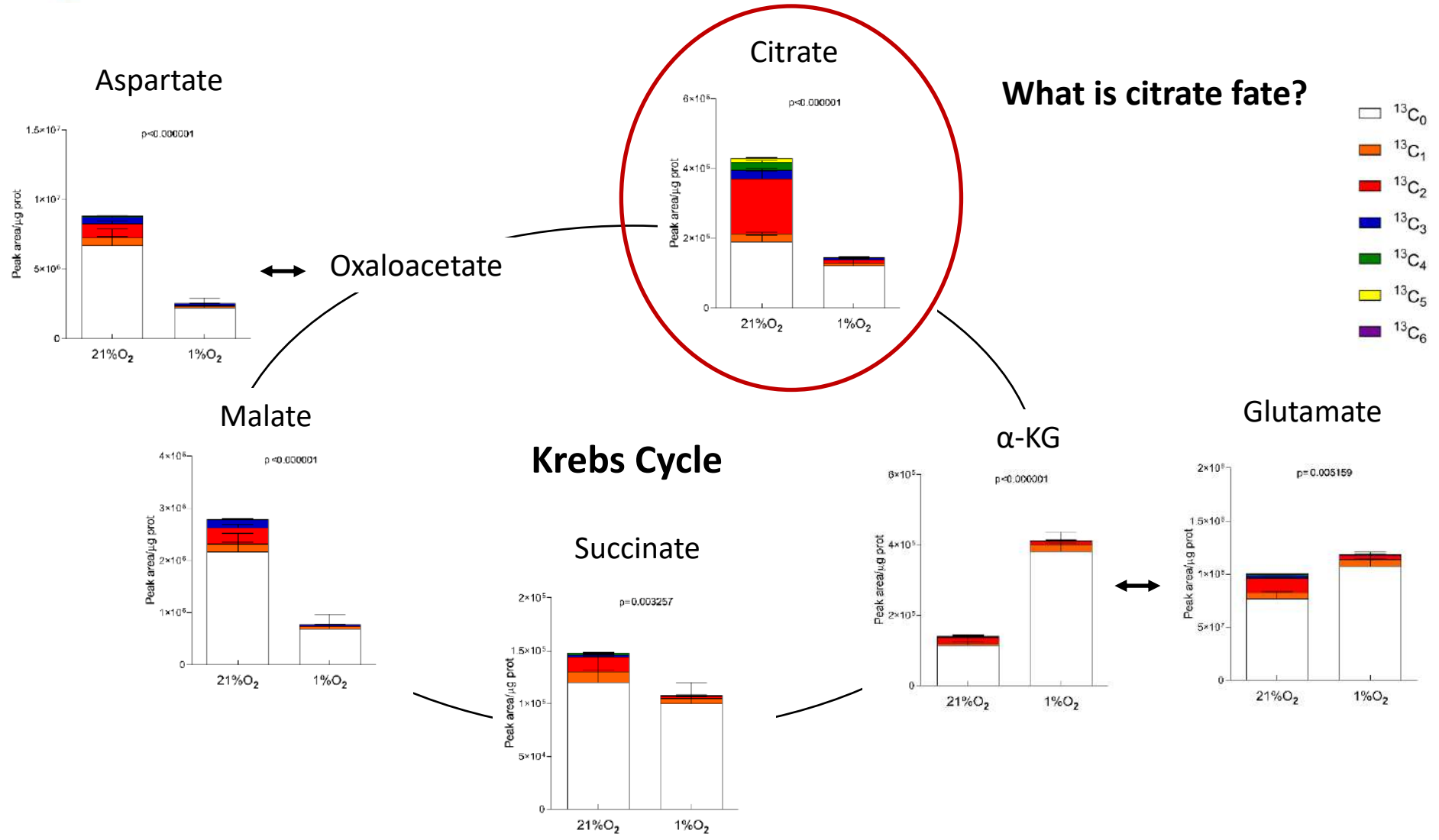




- At both 21% and 1% of O_2 the percentage of glucose converted into lactate is comparable and larger than 60%
- The increased glycolytic flux under hypoxia is not due to a metabolic switch from oxydative phosphorylation (OXPHOS) to glycolytic metabolism
- The energy supply from glucose is given by glycolysis regardless of different oxygen concentrations

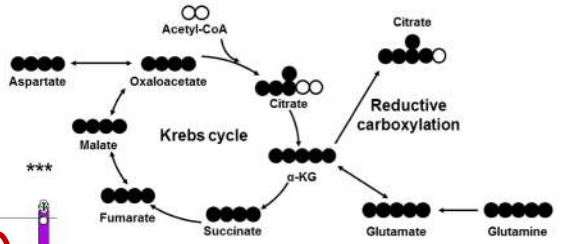
MSCs exhibit aerobic glycolysis, a feature commonly associated to a cancer- and pluripotent-like metabolic phenotypes

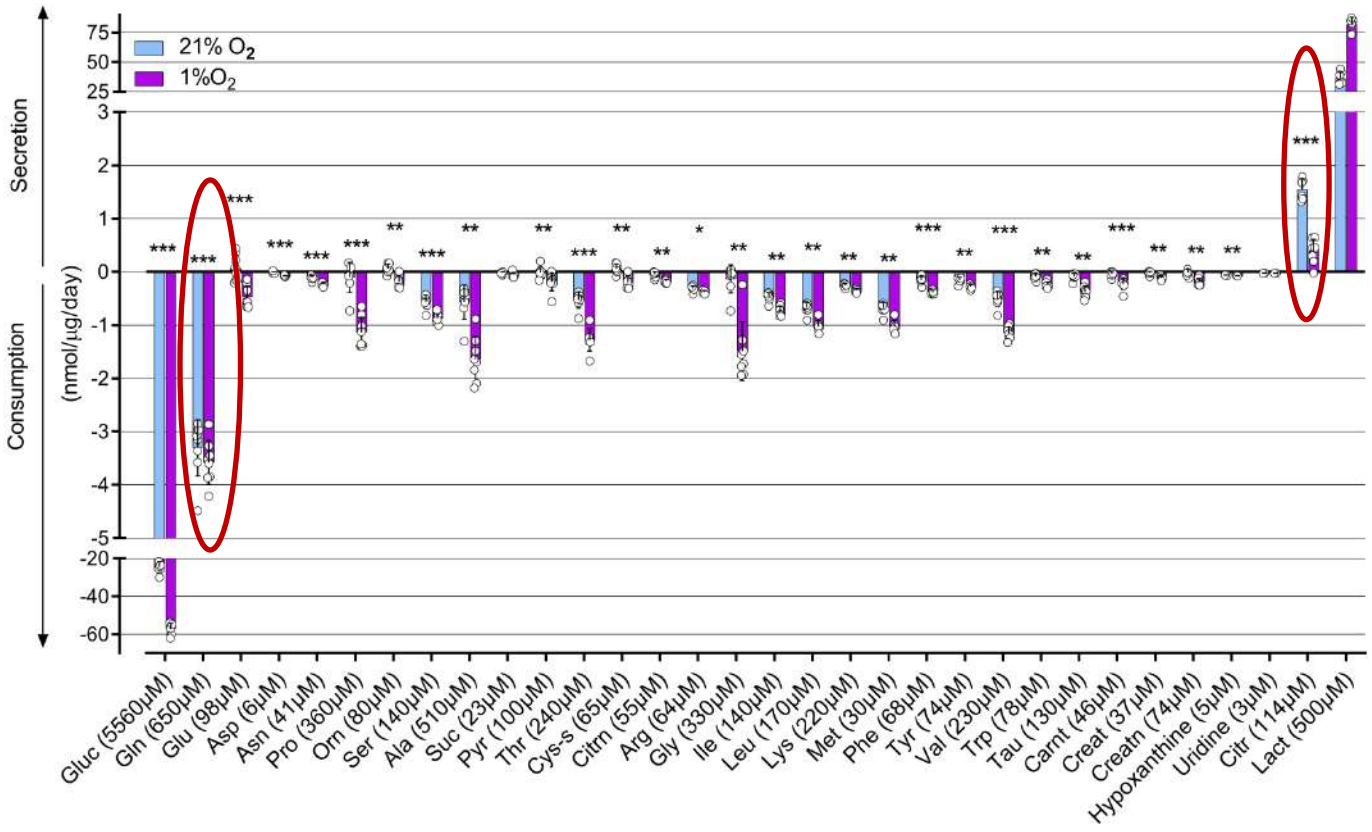
$^{13}C_6$ Glucose tracing



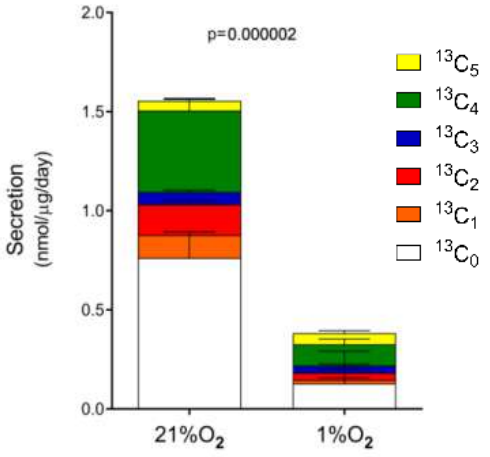
At 21%O_2, glucose-derived carbons are mainly used to produce citrate but no other Krebs intermediate

Citrate secretion



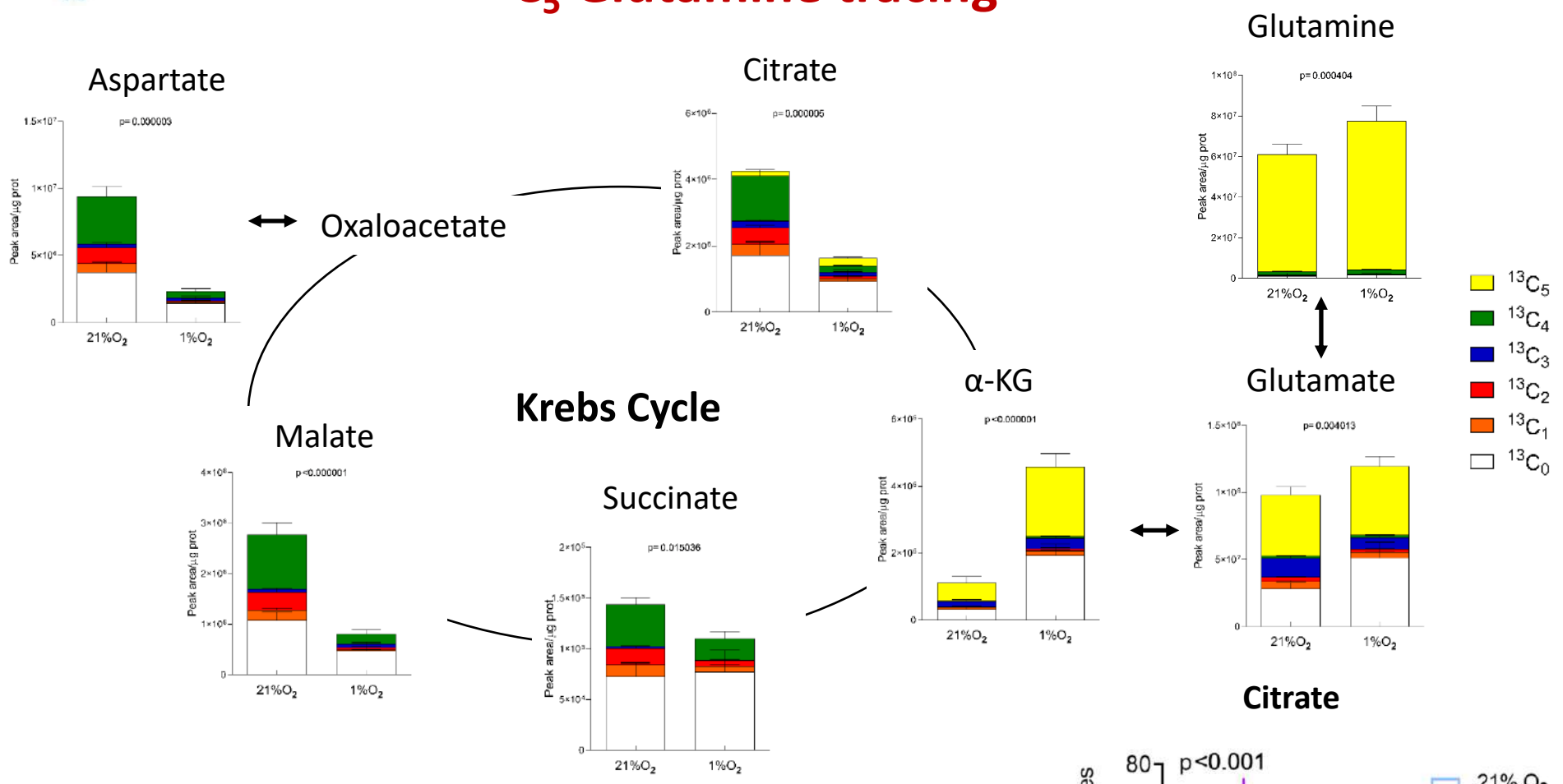


Citrate

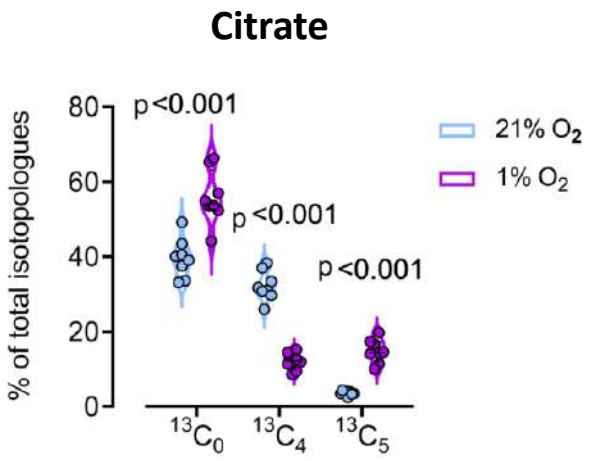


MSCs use extracellular glutamine to synthesize and secrete citrate at both 21% and 1% O_2

$^{13}C_5$ Glutamine tracing

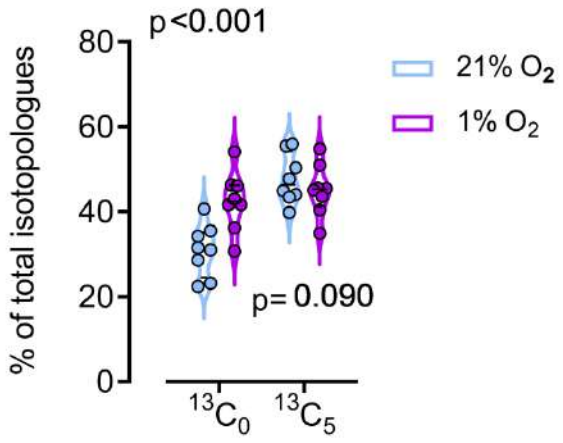


- MSCs exploit extracellular Glutamine for anaplerosis at both 21% and 1% O_2
- At 1% O_2, MSCs use glutamine-derived α-KG to synthesize citrate through reductive carboxylation

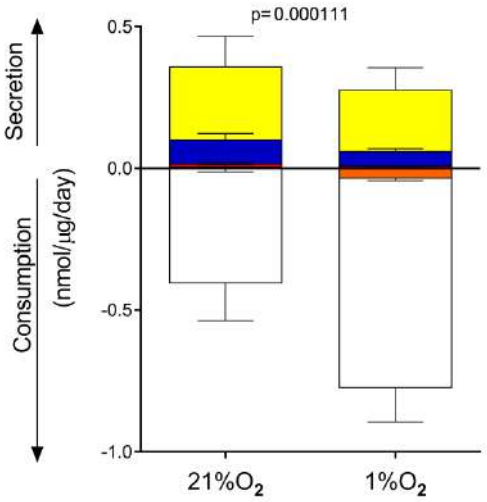


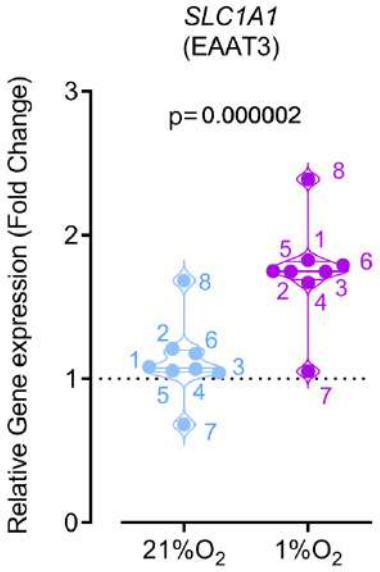
Glutamate metabolism

α-KG

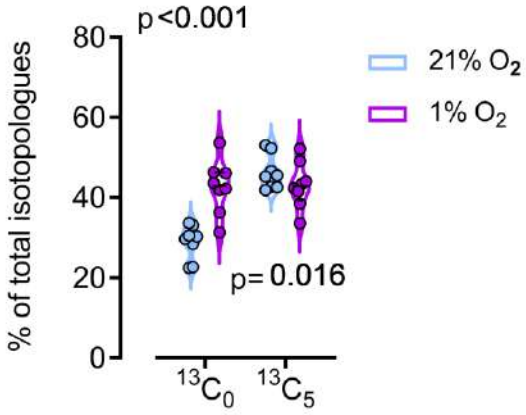


Glutamate uptake





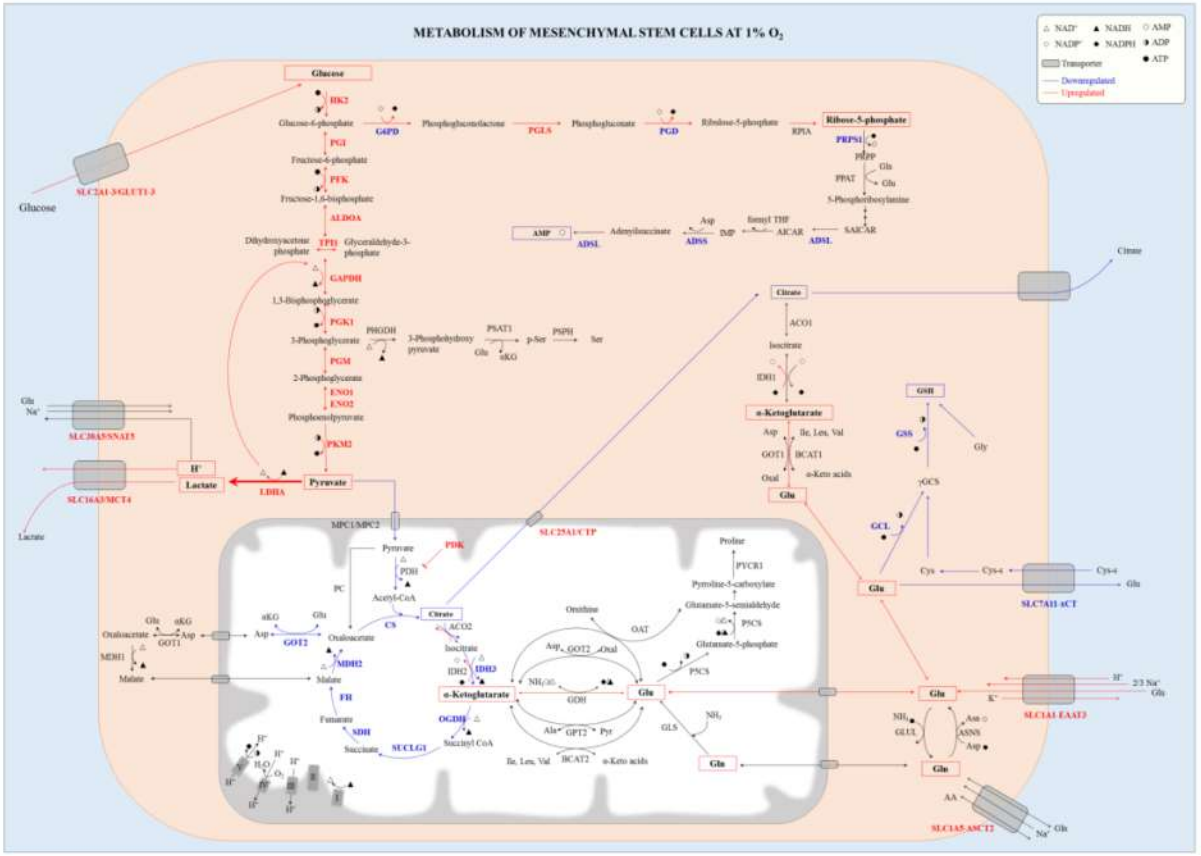
Glutamate



MSCs take up glutamate through the transporter EAAT3 and use the amino acid for anaplerosis and citrate synthesis

A metabolic map of MSCs: work in progress

Low O_2 (closer to *in vivo* conditions)



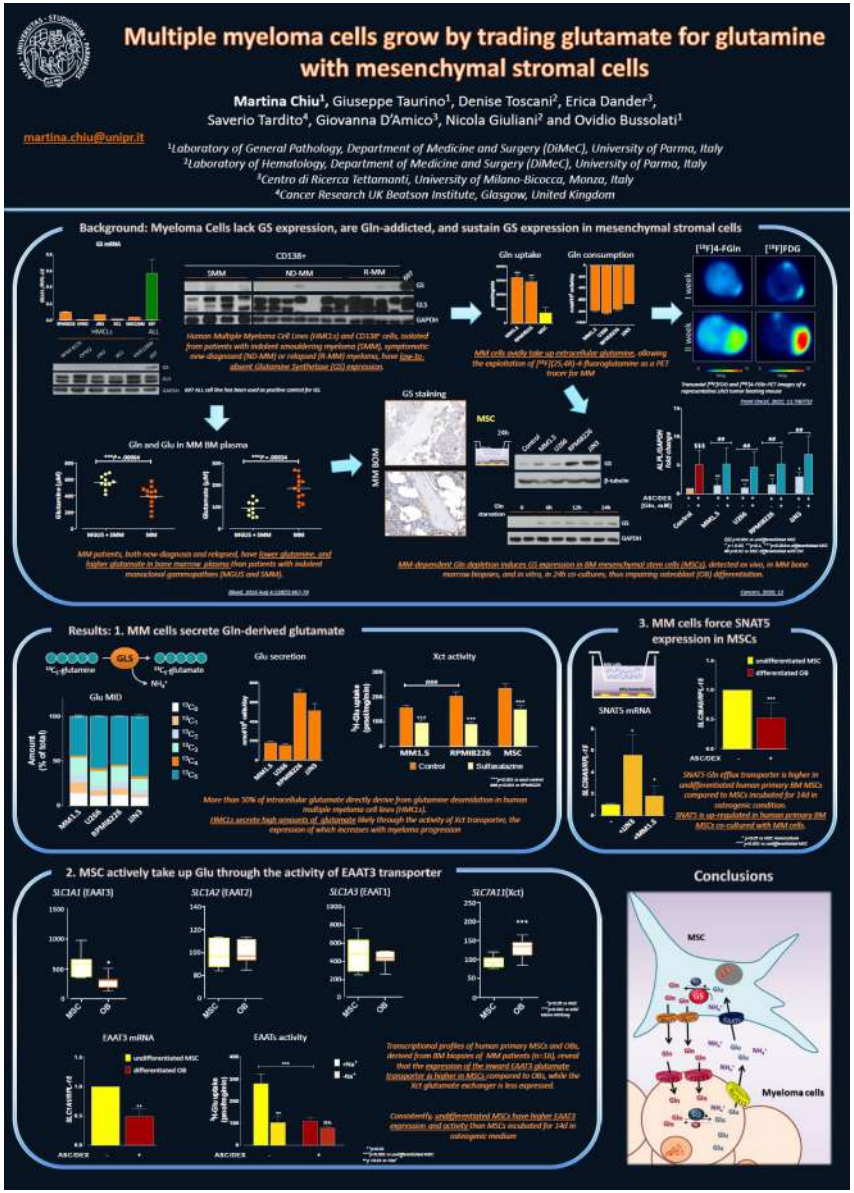
In plasma-like physiological medium Mesenchymal Stem Cells:

- ❑ exhibit **aerobic glycolysis**
- ❑ exploit extracellular glutamine and **glutamate** for anaplerosis and citrate synthesis
- ❑ actively secrete **citrate**

How these MSC metabolic features are involved in normal and neoplastic haematopoiesis?

Acknowledgments

Poster: Dr. Martina Chiu



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