SELECTED OPPORTUNITIES IN IMMUNOLOGY/IMMUNO-ONCOLOGY

New Method to Treat Acidosis Related Diseases

(BIO19221)

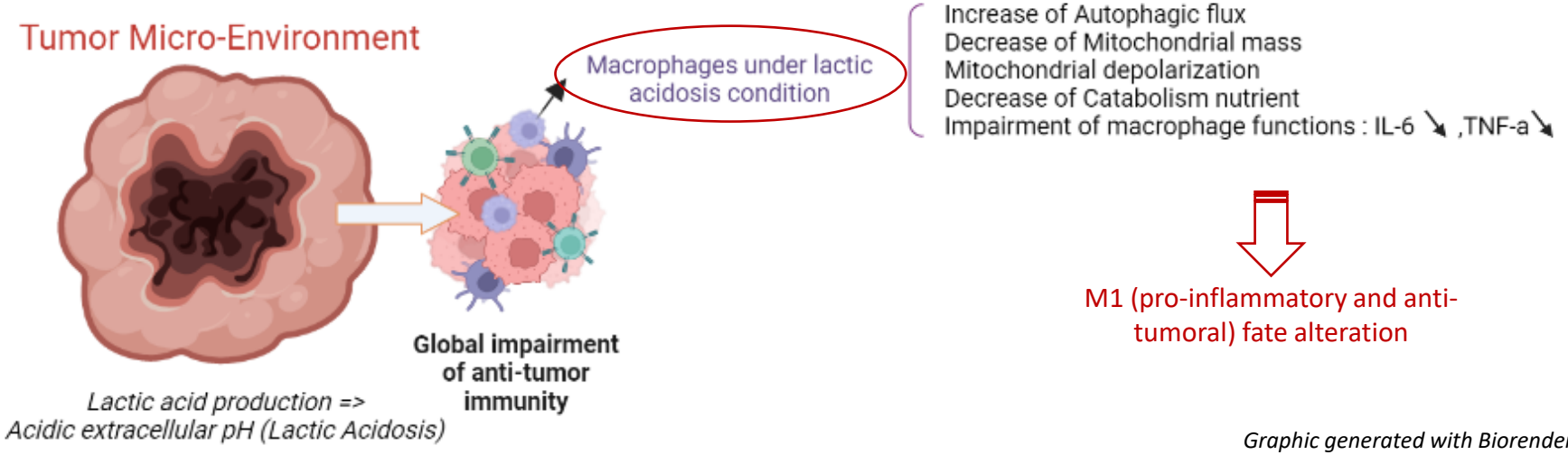
NEW METHOD TO TREAT ACIDOSIS RELATED DISEASES (BIO19221)

Product factsheet

Product

- **Target:** lactic acidosis-induced pseudostarvation
- **Product:** acetoacetate, a natural metabolite originally produced by the liver
- **Application:** acidosis related diseases
- **Rational / POC:**
  - Human macrophages display a reduced mitochondrial mass in lactic acidosis
  - The expression of genes involved in mitochondrial biogenesis is unaffected by lactic acidosis
  - The autophagic flux in macrophages is enhanced during lactic acidosis
  - Macrophages rely on autophagy to survive during lactic acidosis
  - Prolonged lactic acidosis induces mitochondrial depolarization in macrophages, resulting in energetic stress
  - Macrophages display metabolic and cellular changes typical of starving cells during lactic acidosis
  - Lactic acidosis greatly decreases nutrient catabolism
  - The metabolic stress induced by lactic acidosis compromises macrophage functions
  - AcAc prevents lactic acidosis-induced pseudostarvation and mitophagy
- **Patent and publication:**
  - *Acetoacetate protects macrophages from lactic acidosis-induced mitochondrial dysfunction by metabolic reprograming. Nature Communications. 2021*
  - NEW METHOD TO TREAT ACIDOSIS RELATED DISEASES. EP21305941.3

Acidosis (lactic) Impacts Immuno-surveillance in Various Pathologic Contexts
Example in Cancer



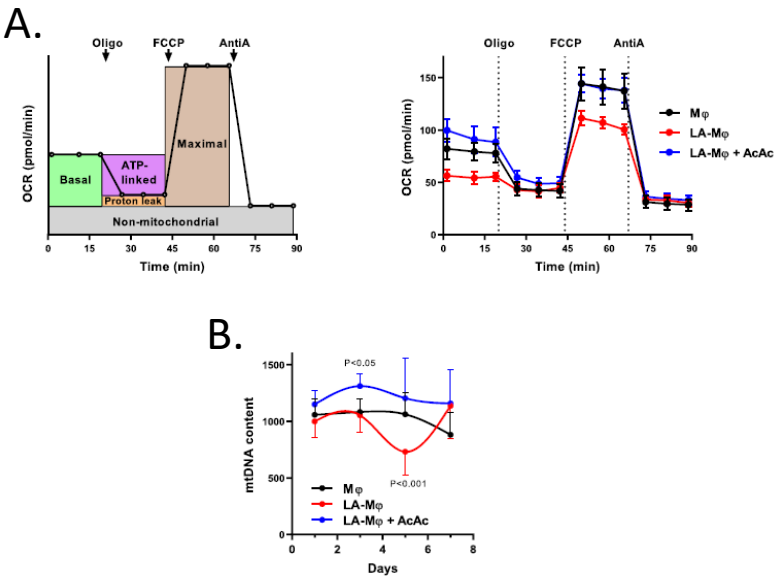
Graphic generated with Biorender

Acidosis and Cancer: from Mechanism to Neutralization. Cancer Metastasis Rev. 2020.
Tumour acidosis: from the passenger to the driver's seat. Nat Rev Cancer. 2017.
Lactic Acid: No Longer an Inert and End-Product of Glycolysis. Physiology. 2017.

NEW METHOD TO TREAT ACIDOSIS RELATED DISEASES (BIO19221)

Rational 1 : AcetoAcetate Restore Mitochondrial Mass and Autophagic Flux in Macrophages under LA Condition

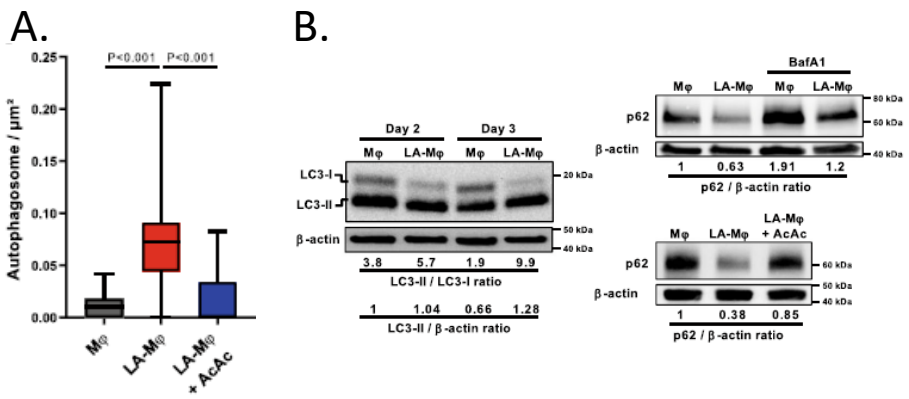
Sustained acidosis affects macrophage mitochondrial mass



Monocytes were polarized into macrophages in the absence (Mϕ) or presence of lactic acid (LA-Mϕ), lactic acid and acetoacetate (LA-Mϕ + AcAc), sodium lactate (Lactate-Mϕ), or under acidosis (HCl-Mϕ).

- **A.** Monitoring of oxygen consumption rate (OCR) in day 4 macrophages following a sequential addition of oligomycin (oligo), carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP), and antimycin A (AntiA).
- **B.** Quantification of mitochondrial DNA (mtDNA) copy number by qPCR from day 1 to day 7 (mean ± SD; n =10).

Human macrophages display upregulation of autophagic flux during lactic acidosis



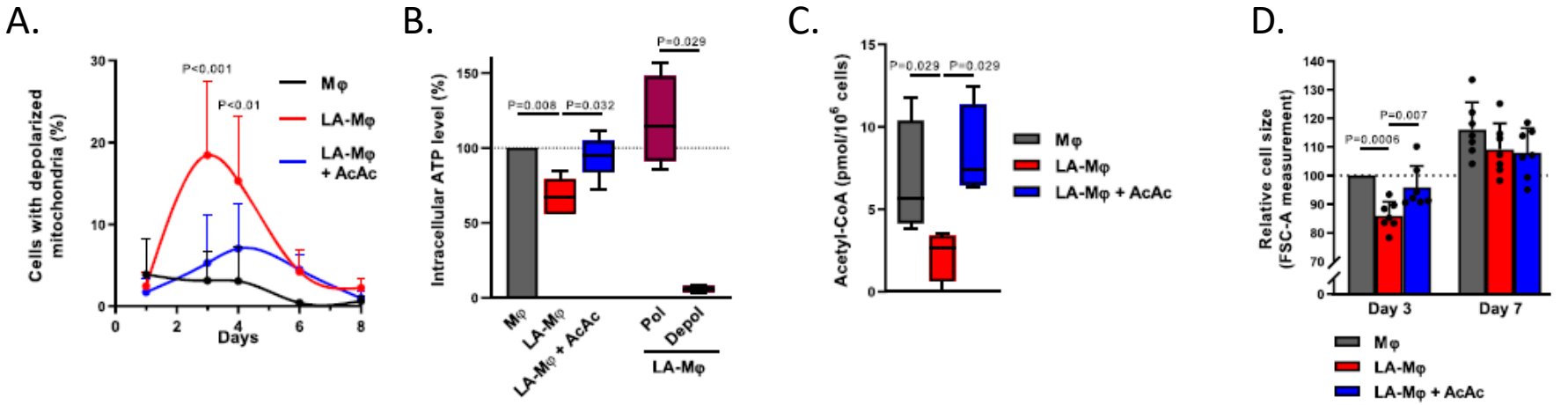
Monocytes were polarized without lactic acid (Mϕ), with lactic acid (LA-Mϕ) or with lactic acid and acetoacetate (LA-Mϕ + AcAc).

- **A.** Quantification of autophagic vesicles per cell on day 3.
- **B.** Western blotting analysis of LC3-I, LC3-II, p62, and β-actin in day 3 Mϕ, in the presence or absence of bafilomycin A1 (BafA1) or AcAc. The LC3-II/LC3-I, LC3-II/β-actin, and p62/β-actin band intensity ratios are indicated

NEW METHOD TO TREAT ACIDOSIS RELATED DISEASES (BIO19221)

Rational 2 : AcetoAcetate Restore Mitochondrial Polarization and Metabolic Fitness of Macrophages under LA Condition

Macrophages in conditions of lactic acidosis display mitochondrial depolarization and characteristics typical of starving cells.



Monocytes were polarized into macrophages in the absence (Mϕ) or presence of lactic acid (LA-Mϕ), lactic acid and acetoacetate (LA-Mϕ + AcAc), sodium lactate (Lactate-Mϕ), or under acidosis (HCl-Mϕ).

- **A.** Cells with depolarized mitochondrial membrane potential (ΔΨm) were analyzed by flow cytometry using MitoTracker Green and MitoTracker Deep Red probes at the indicated time point
- **B.** Intracellular ATP levels were measured in a semiquantitative assay.
- **C.** Intracellular acetyl-CoA levels determined on day 3.
- **D.** Cell size was measured on day 7, by determining the relative cell size by flow cytometry with the FSC-A parameter.

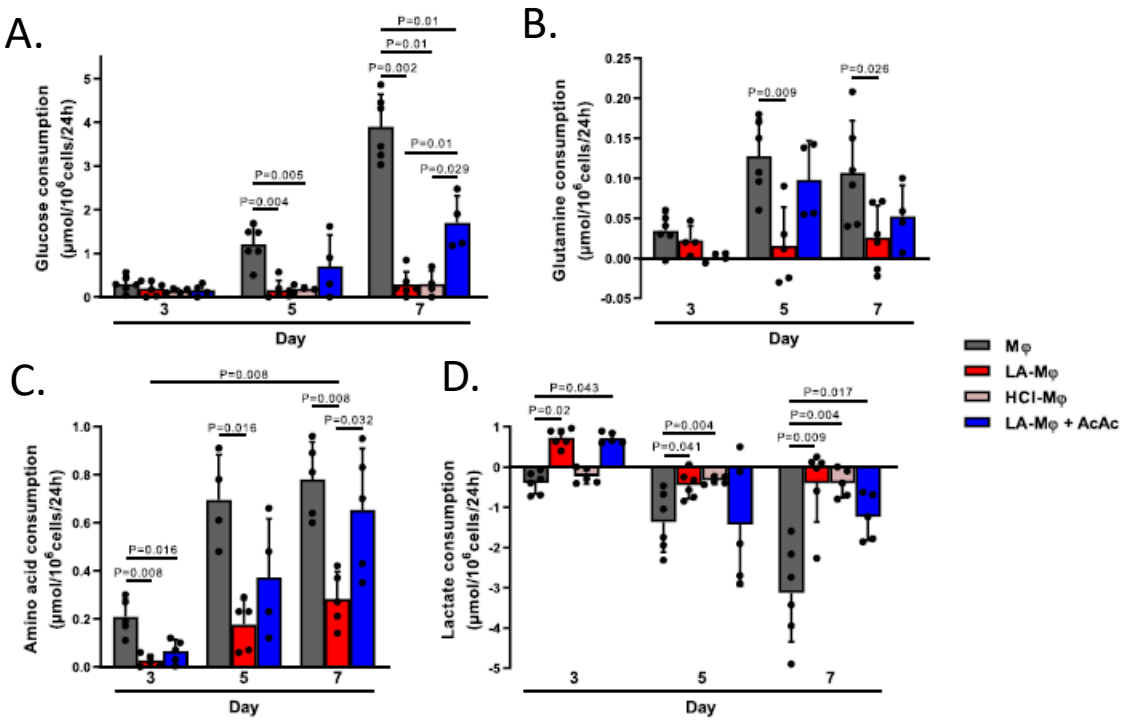
NEW METHOD TO TREAT ACIDOSIS RELATED DISEASES (BIO19221)

Rational 2 bis : AcetoAcetate Restore Metabolic Fitness of Macrophages under LA Condition

Lactic acidosis is associated with pseudostarvation.

Monocytes were polarized into macrophages in the absence (Mϕ) or presence of lactic acid (LA-Mϕ), lactic acid and acetoacetate (LA-Mϕ + AcAc), sodium lactate (Lactate-Mϕ), or under acidosis (HCl-Mϕ).

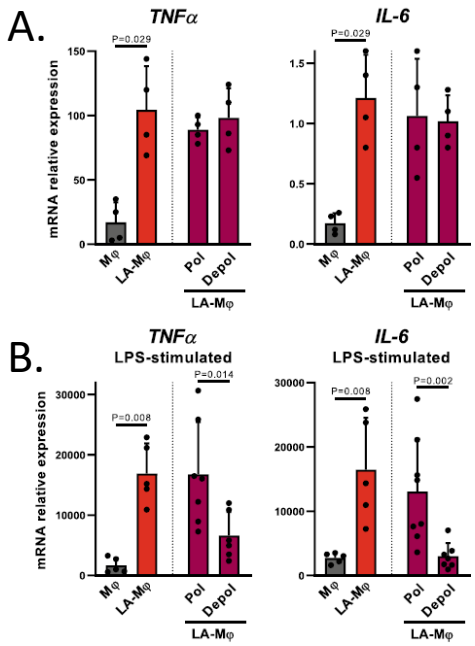
- Glucose (**A.**), glutamine (**B.**), free L-amino acids (**C.**), and lactate (**D.**) were quantified at days 3, 5, and 7 in cell culture supernatants of Mϕ, LA-Mϕ, LA-Mϕ + AcAc, and HCl-Mϕ. Results are expressed in µmol/10^6 cells/24 h, with positive values for consumption and negative values for production.



NEW METHOD TO TREAT ACIDOSIS RELATED DISEASES (BIO19221)

Rational 3 : AcetoAcetate Restore Cytokines Profiling of Macrophages under LA Condition

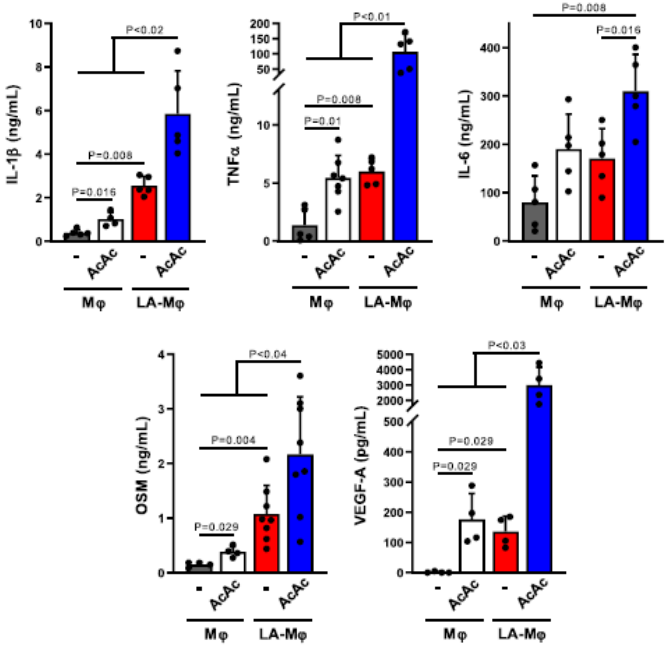
Relative expression of cytokine mRNAs by LA-Mϕ with depolarized mitochondria.



Monocytes were polarized into macrophages by incubation in the absence (Mϕ) or presence of lactic acid (LA-Mϕ) for 3 days. LA-Mϕ with and without depolarized mitochondrial membranes ("Depol" and "Pol" populations, respectively) were sorted by flow cytometry with MitoTracker probes.

- Cells were unstimulated (**A.**) or stimulated with LPS for 3 h (**B.**) and the levels of TNFα and IL-6 mRNA were assessed by RT-qPCR. The results were expressed as mRNA levels relative to those for the housekeeping gene RPS18.

Impact of AcAc on cytokine secretion by LA-Mϕ.

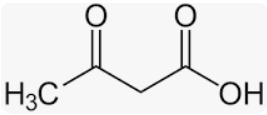


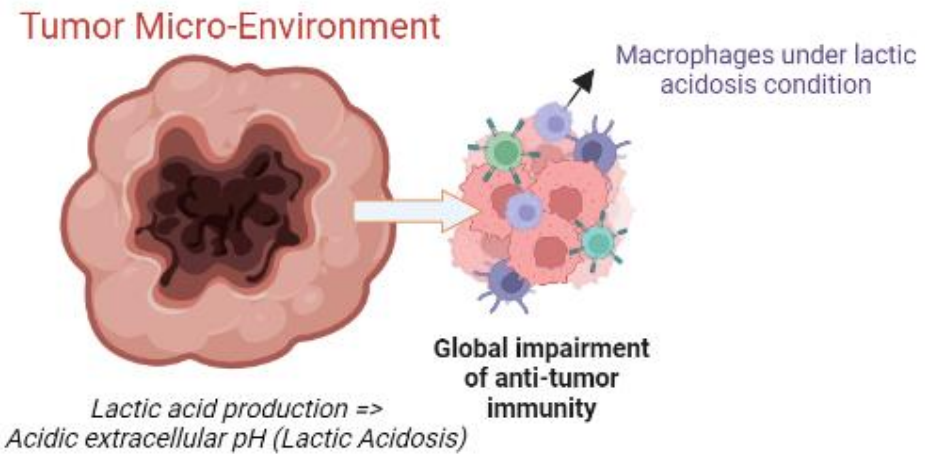
Monocytes were differentiated into macrophages in the absence (Mϕ) or presence of lactic acid (LA-Mϕ), with or without acetoacetate (AcAc).

- On day 5, cells were stimulated for 16 h with LPS and IL-1β, TNFα, IL-6, oncostatin M (OSM), and VEGF-A were quantified by ELISA in the supernatants

Acetoacetate is a Candidate to Overcome Macrophages Immune Alterations Acidosis-related Example in Cancer

+AcetoAcetate



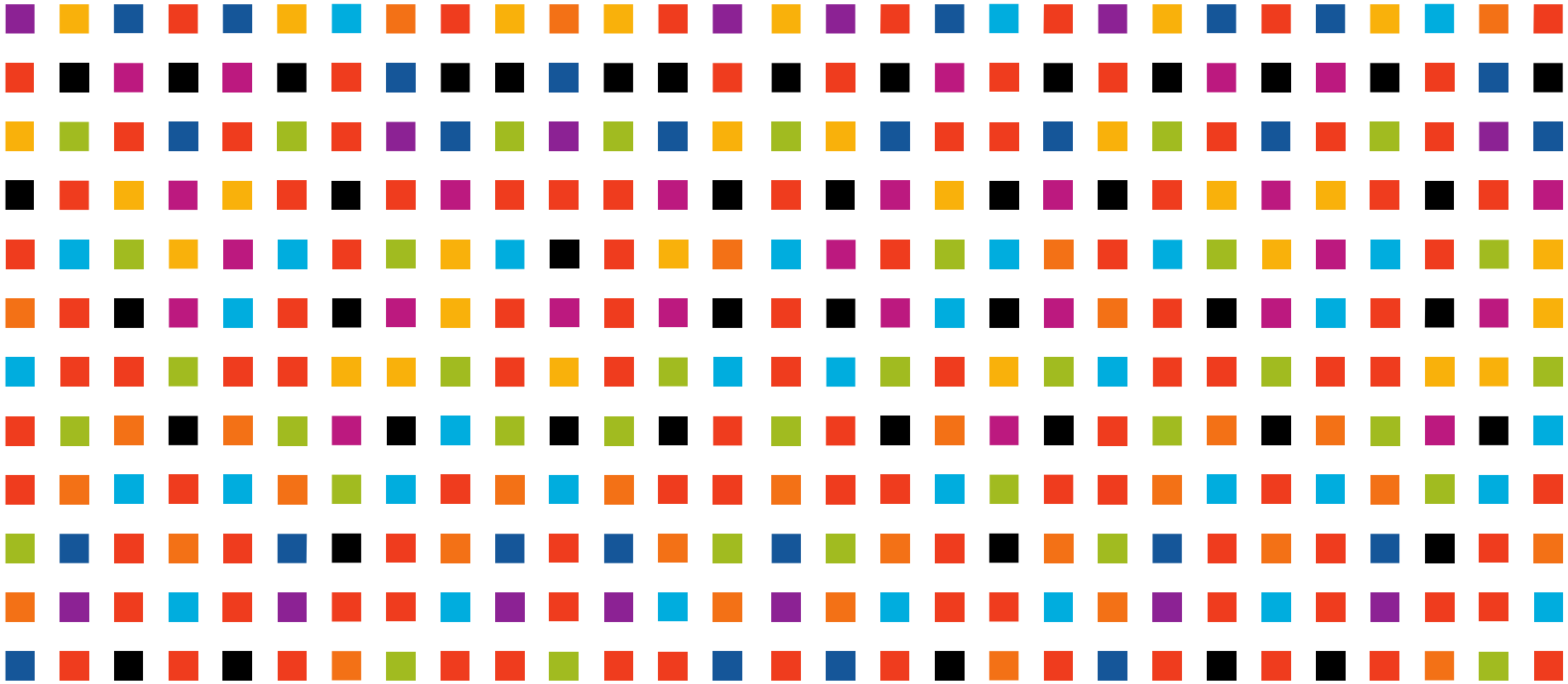


- Restoration of Autophagic flux
- Restoration of Mitochondrial mass
- Mitochondrial polarization
- Restoration of Catabolism nutrient
- Restoration of macrophage functions : IL-6 ↗ ,TNF-a ↗

M1 (pro-inflammatory and anti-tumoral) fate restoration

Graphic generated with Biorender

Acidosis and Cancer: from Mechanism to Neutralization. Cancer Metastasis Rev. 2020.
Tumour acidosis: from the passenger to the driver's seat. Nat Rev Cancer. 2017.
Lactic Acid: No Longer an Inert and End-Product of Glycolysis. Physiology. 2017.



NATHAN.POMORSKI@INSERM-TRANSFERT.FR