FONDAZIONE IRCCS ISTITUTO NAZIONALE DEI TUMORI

UNIVERSITÀ DEGLI STUDI DI MILANO

IFOM

Alimentazione e carcinoma mammario: quali dati dalla ricerca?

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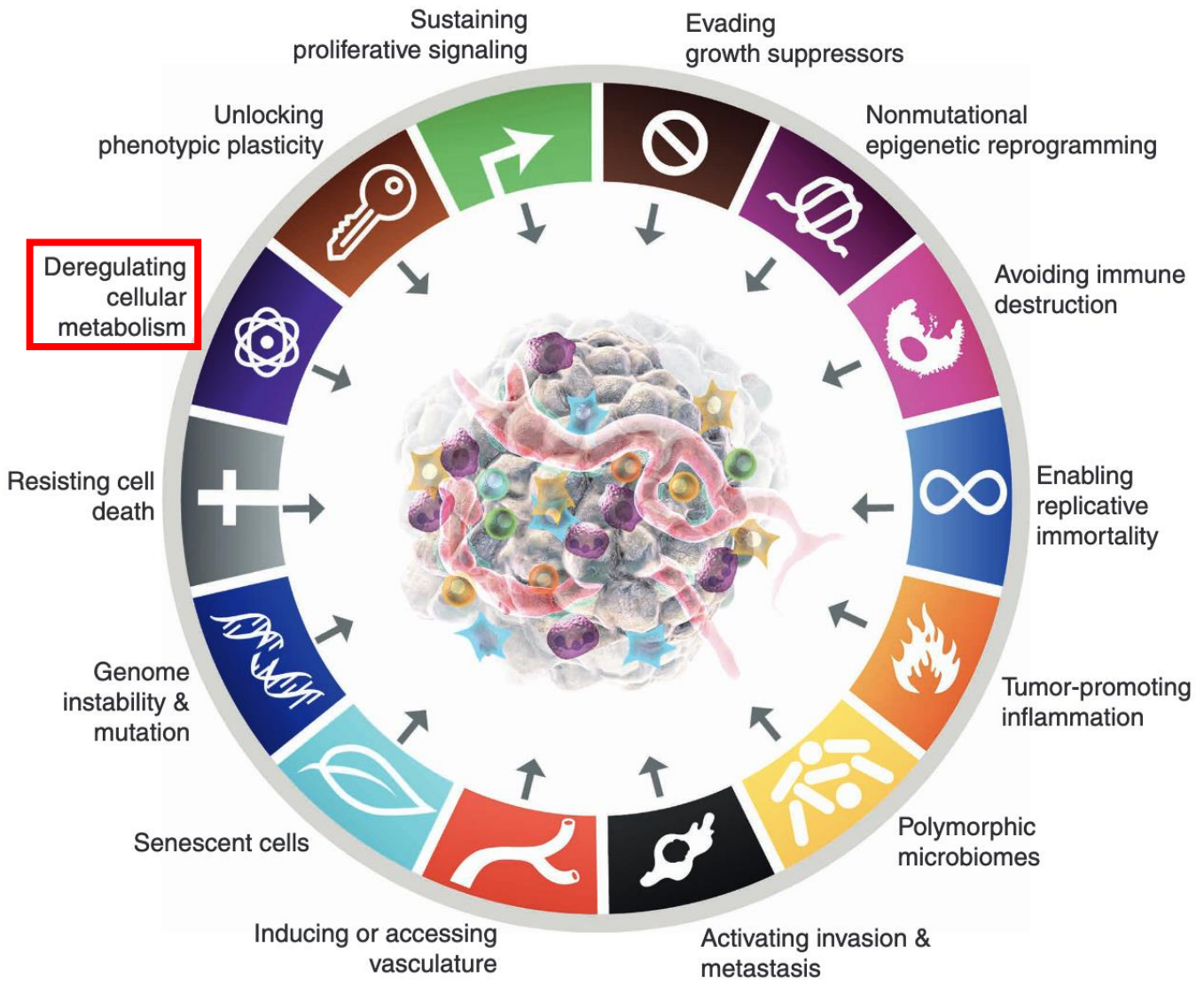
Disclosures

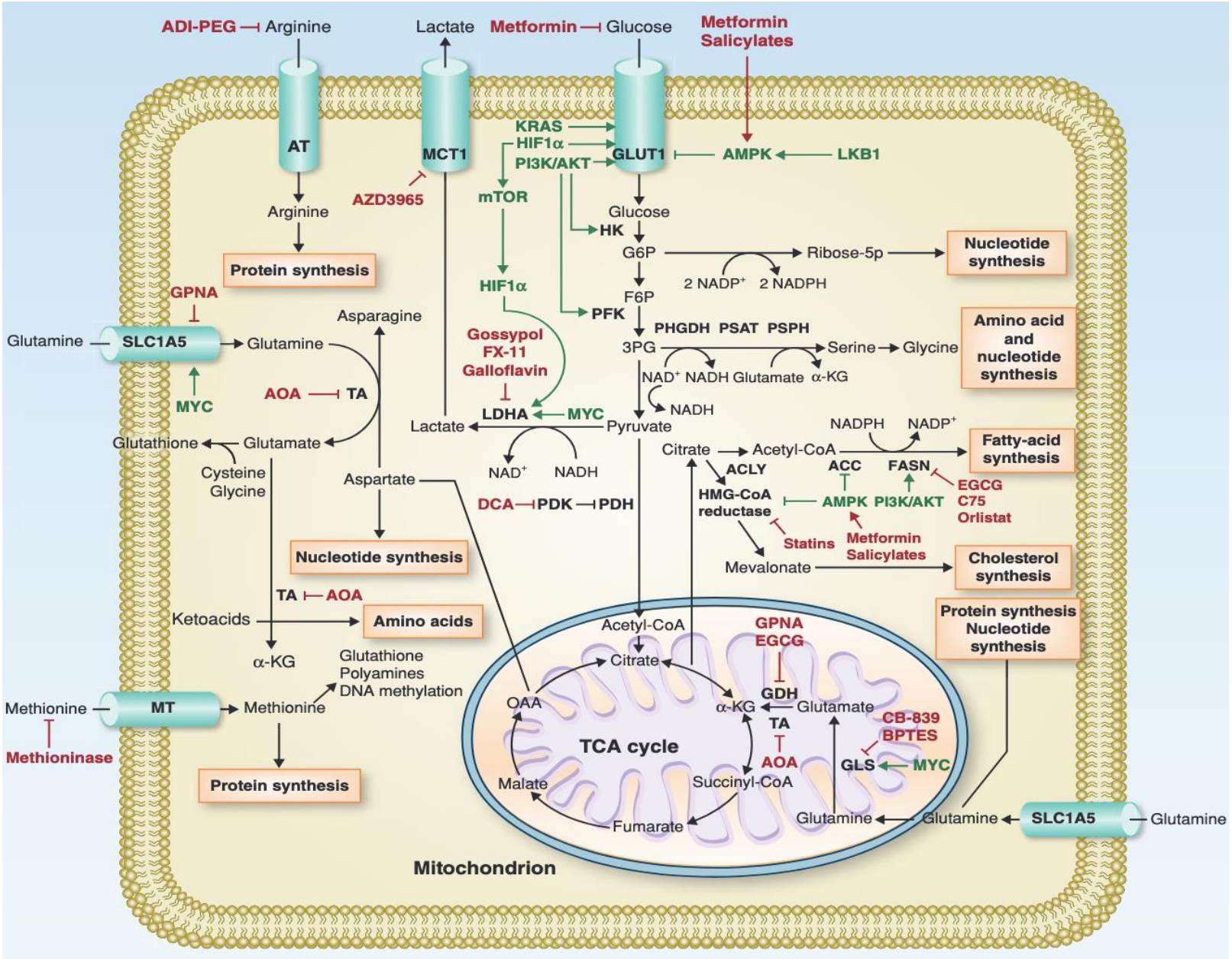
- **Role in advisory boards**: Novartis, Eli Lilly, Daiichi Sankyo, Pfizer, Menarini Stemline, Astra Zeneca
- **Consultancy**: Eli Lilly, Novartis
- **Honoraria as a Speaker**: Eli Lilly, Novartis, Pfizer, MSD, Menarini Stemline, Daiichi Sankyo, Astra Zeneca, Istituto Gentili, Accademia Nazionale di Medicina
- **Grants (to the Institution)**: AIRC, ERC, AIRC 5x1000, Ministero della Salute, Roche, Giuliani Foundation, Daiichi Sankyo, Scientific Directorate of Fondazione IRCCS Istituto Nazionale dei Tumori

Metabolic reprogramming: an established hallmark of cancer



Hanahan D. Cancer Discov 2022

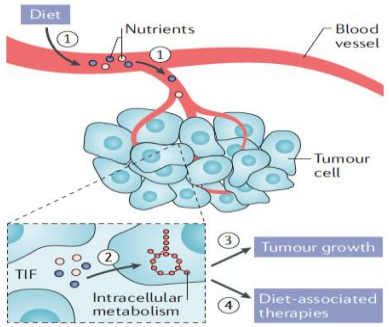
The crosstalk between oncogenic and metabolic pathways



Vernieri C et al Cancer Discov 2016

Different approaches to target metabolic reprogramming in human cancers

Dietary interventions



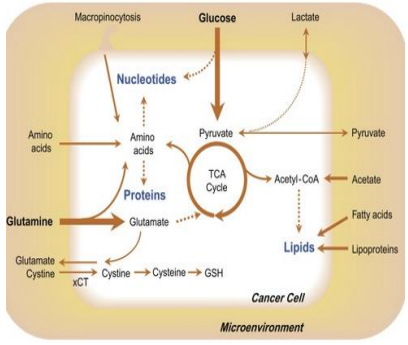
Extracellular metabolic environment (e.g. metabolite availability)

Pharmacologic inhibition of oncogenic pathways

Pharmacologic inhibition of metabolic pathways

Tumor genetics (e.g. *KRAS, c-MYC*)

Metabolic dependencies (HK, PFK, FASN)



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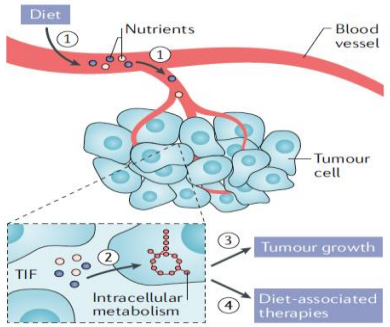

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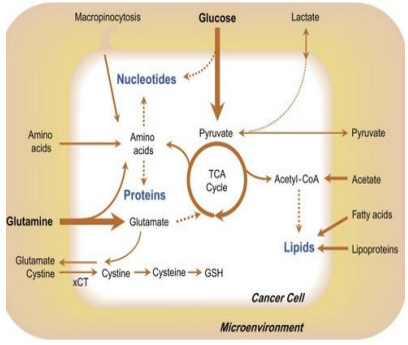


Different nutritional interventions

- Nutrient deprivation

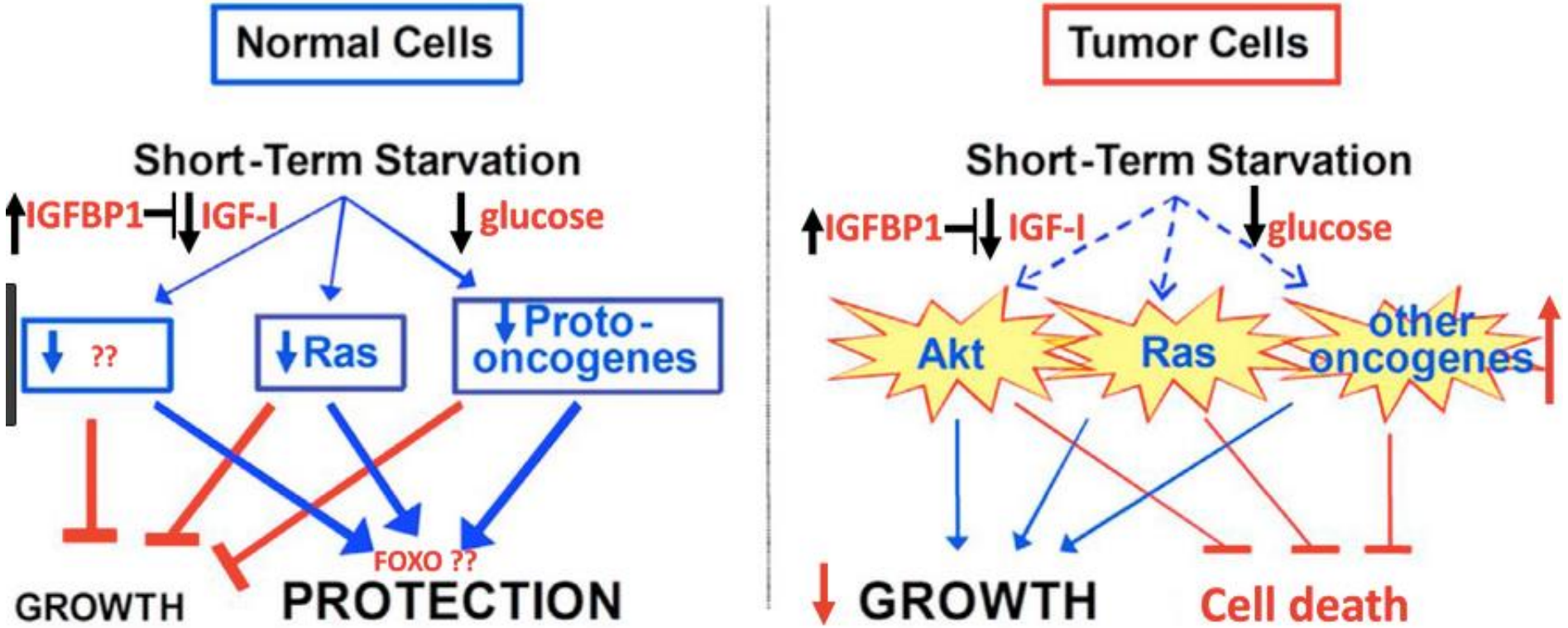
- Nutrient supplementation

Different nutritional interventions

- **Nutrient deprivation**

- Nutrient supplementation

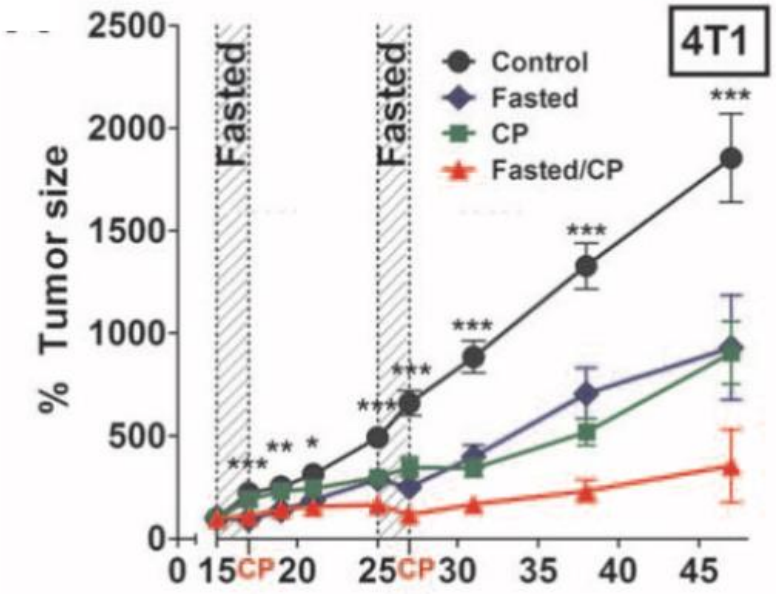
Differential stress resistance to nutrient starvation of normal cells vs. cancer cells: the currently accepted model

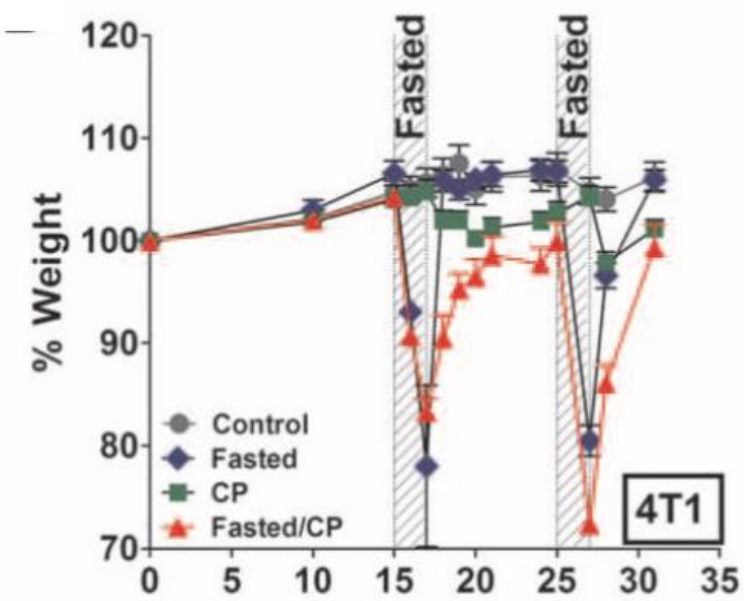


Longo VD and Mattson MP. Cell Metab 2014
Lee C et al. Sci Transl Med 2012
Guevara-Aguirre J et al. Sci Transl Med 2011
Lee C et al. Cancer Res 2010

Cyclic fasting improves the in vivo effects of cytotoxic chemotherapy in murine TNBC models, and it simultaneously protects normal tissues

Ortothopic, syngeneic murine TNBC model





Lee et al. Sci Trans Med 2012

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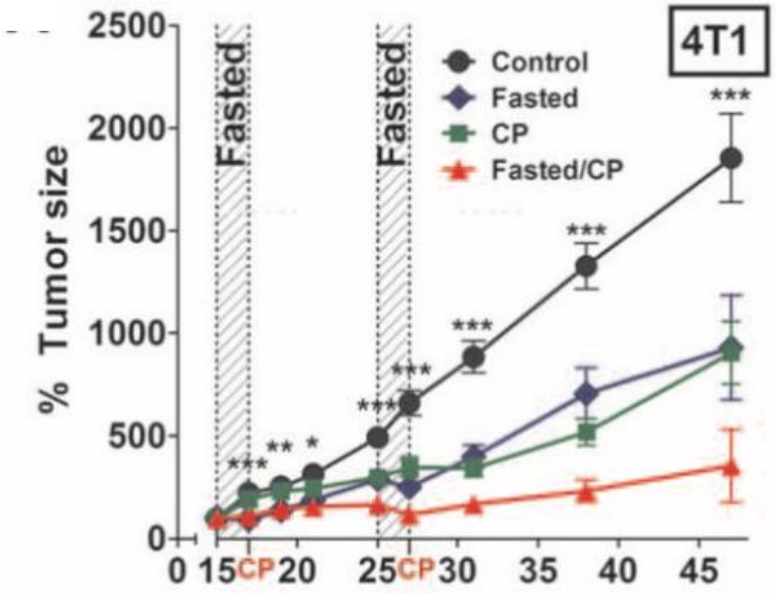
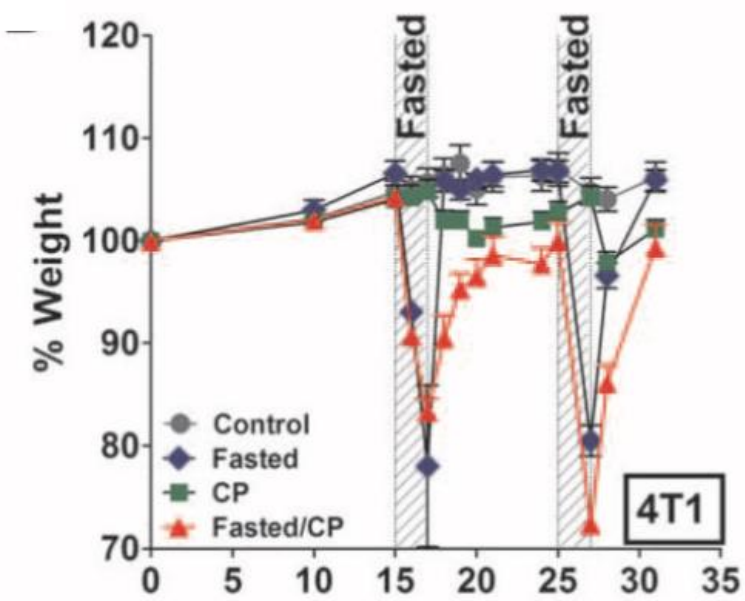
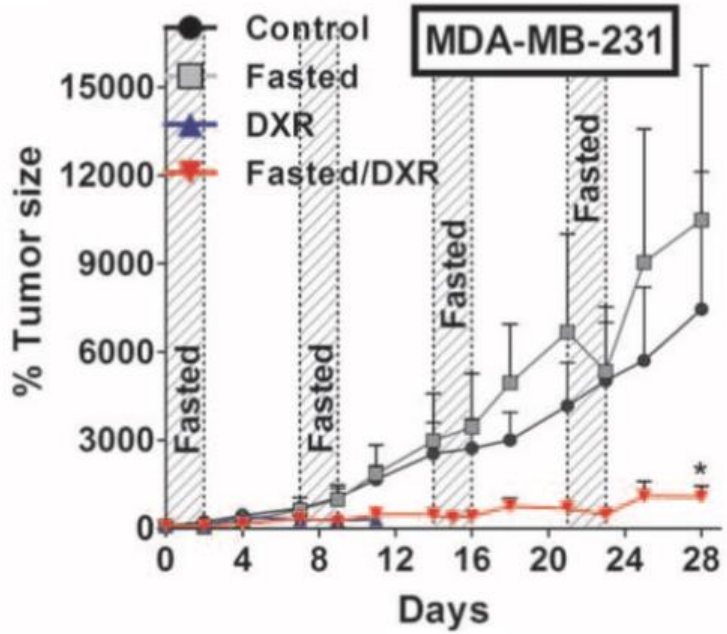




Ortothopic mouse xenografts of human TNBC cells



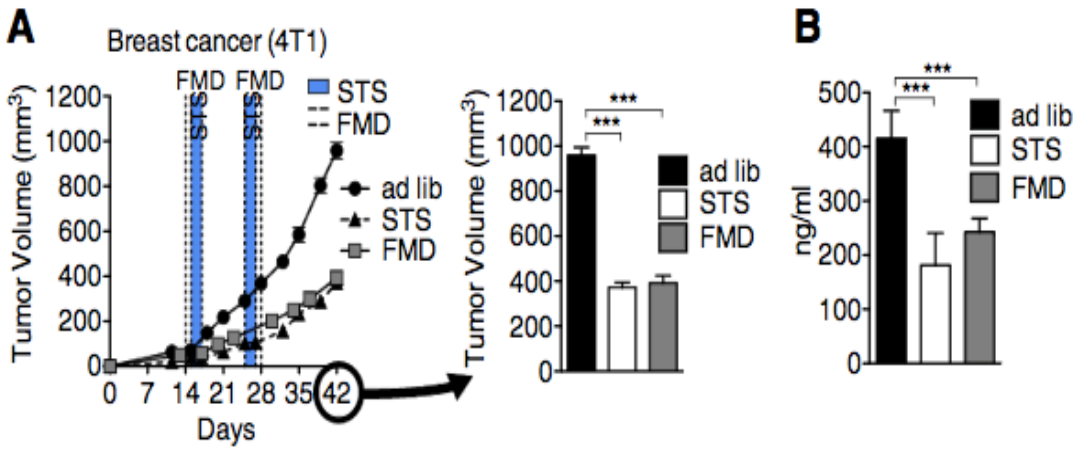
Lee et al. Sci Trans Med 2012

Fasting-mimicking diets (FMDs)

"Any diet that is specifically composed to induce the metabolic effects of fasting while allowing for a potentially higher caloric intake, including solid foods. It usually refers to aplant-based, calorie-restricted diet with a maximum of approximately 1000 Kcal per day that lasts 3 to 7 days"

International Consensus; Koppold D et al. Cell Metabolism 2024

FMD is as effective as water-only fasting as an anticancer treatment



Di Biase S. et al. Cancer Cell. 2016

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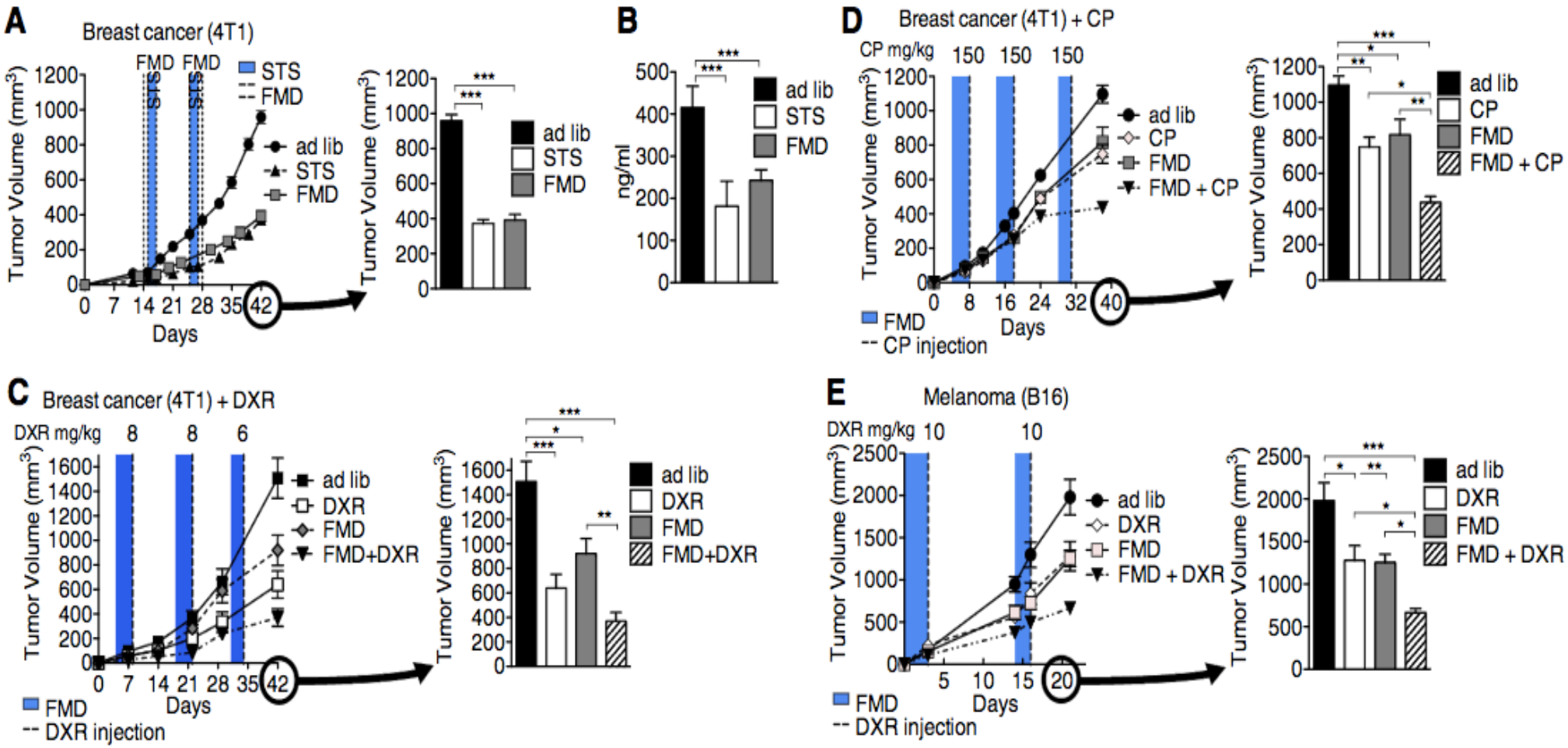
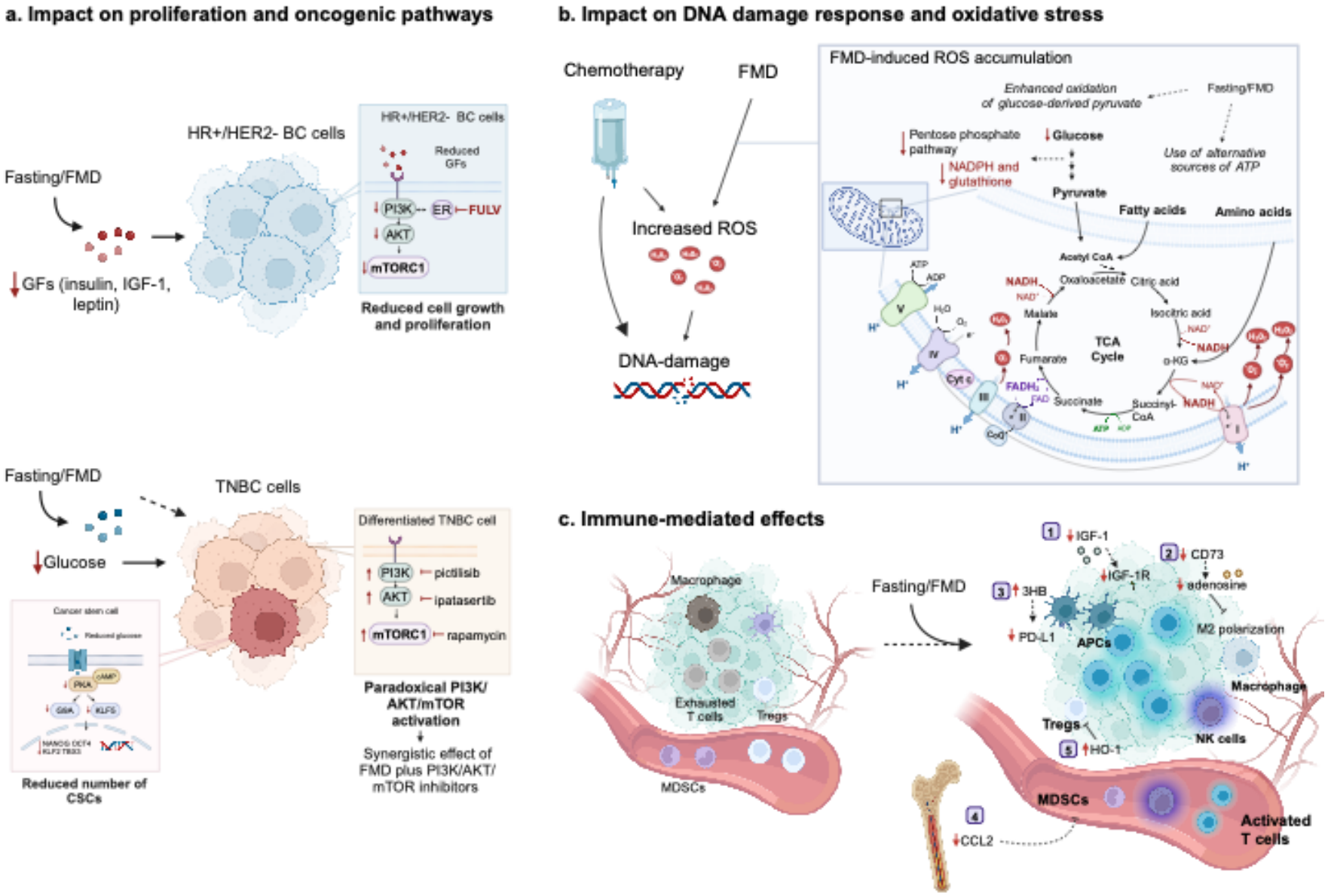


Di Biase S. et al. Cancer Cell. 2016

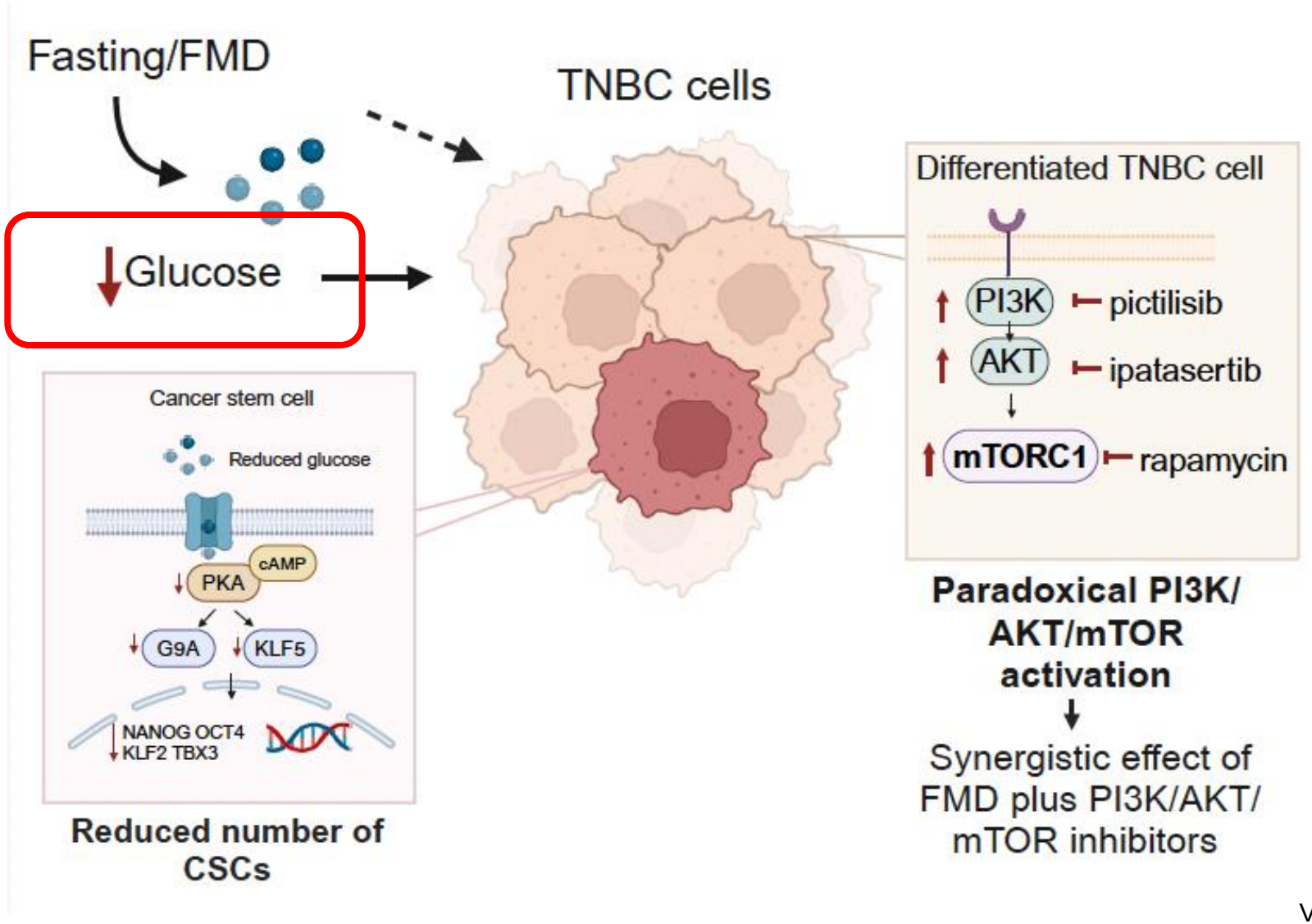
What are the metabolic and mechanistic determinants of fasting/FMD antitumor effects?

Tumor cell autonomous and immune system mediated mechanisms of FMD antitumor effects



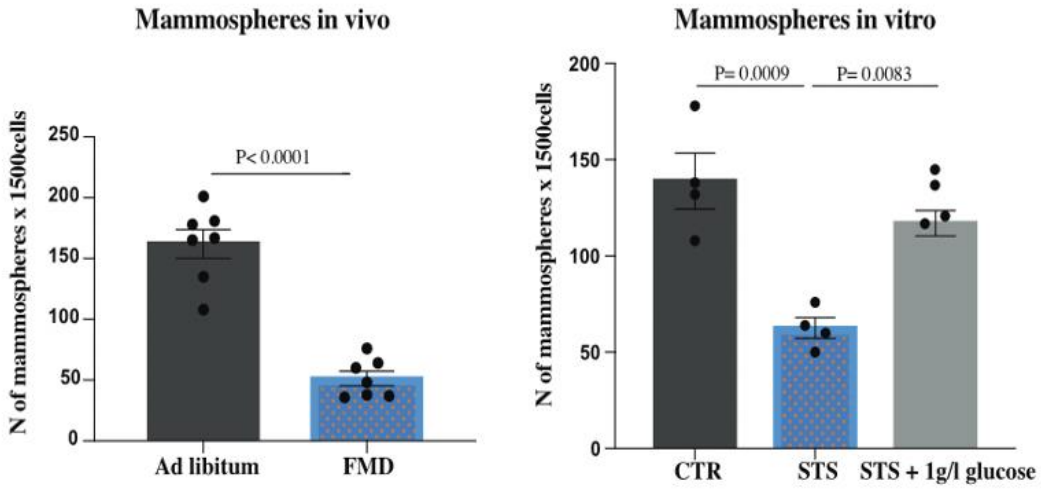
Vernieri C et al. Cell Metab 2024

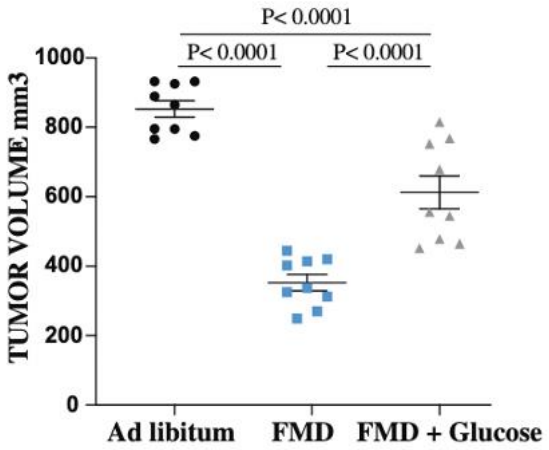
While reducing TNBC CSCs, cyclic FMD makes more differentiated cancer cells exquisitely sensitive to pharmacologic inhibition of the PI3K/AKT/mTORC1 axis

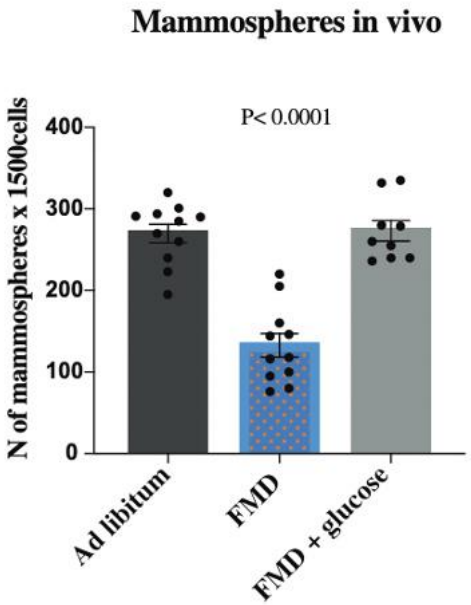


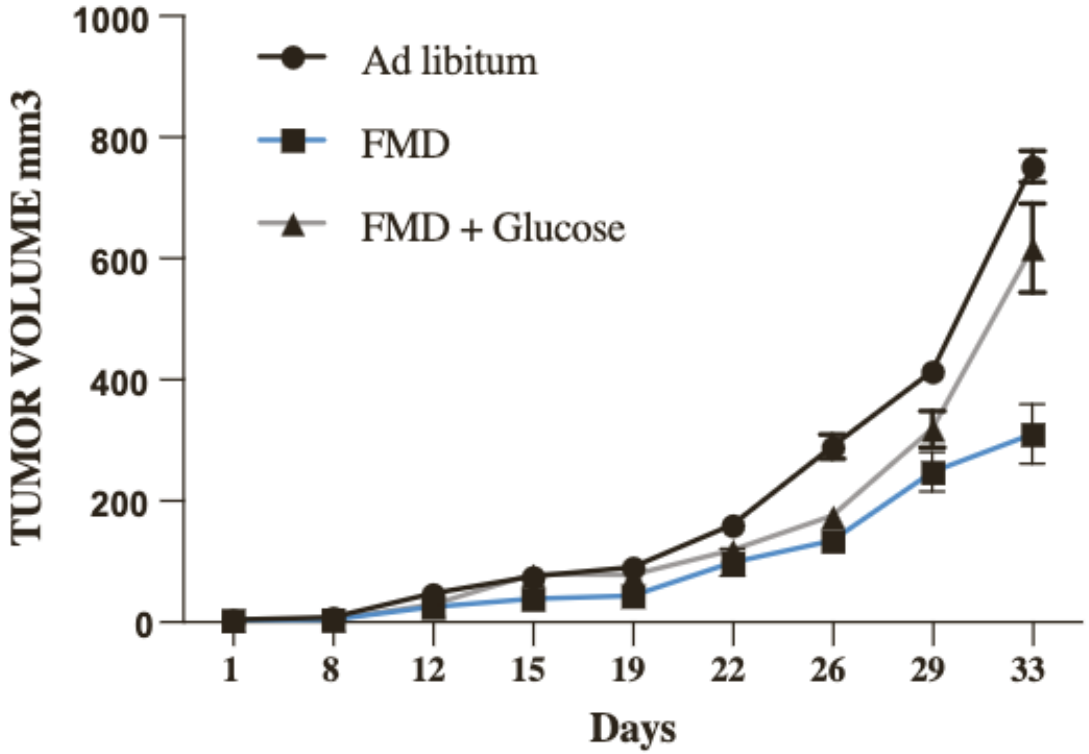
Vernieri C et al. Cell Metab 2024

Cyclic FMD reduces intratumor TNBC cancer stem cells (CSCs) as a result or lowered extracellular blood glucose concentration



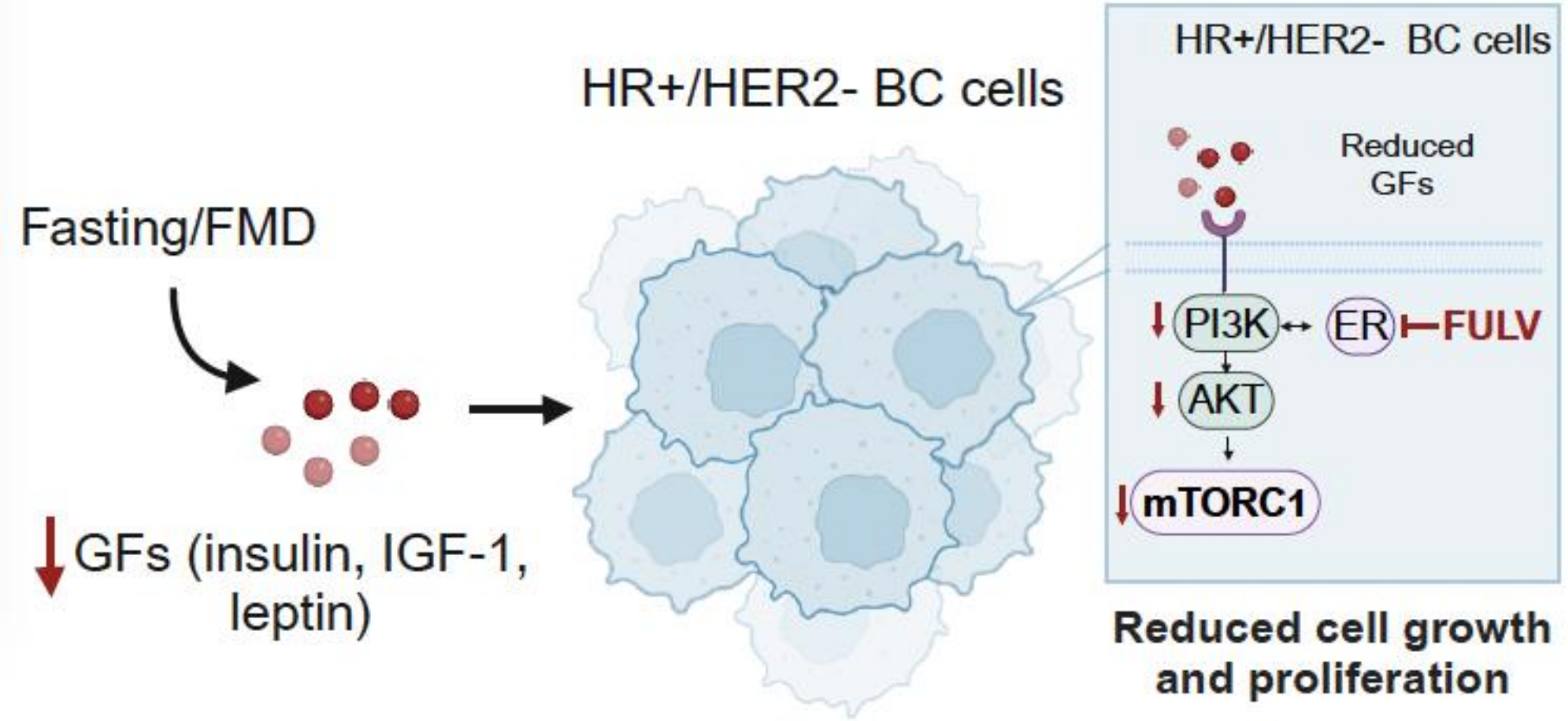






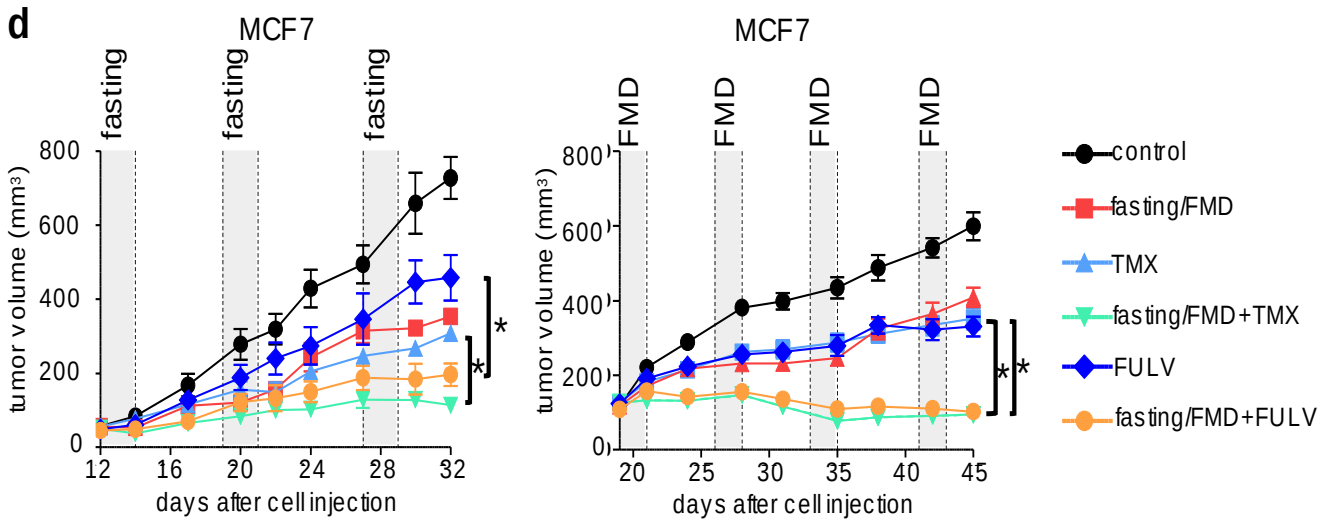
Salvadori G et al. Cell Metab 2021

Cyclic FMD is a non pharmacological inhibitor of the PI3K/AKT/mTORC1 axis, which is crucially involved in acquired HR+/HER2- BC cell resistance to endocrine therapies plus CDK4/6 inhibitors



Vernieri C et al. Cell Metab 2024

Endocrine therapy synergizes with cyclic fasting/FMD in mouse models of HR+/HER2- BC



Caffa I, Spagnolo V et al. Nature 2020

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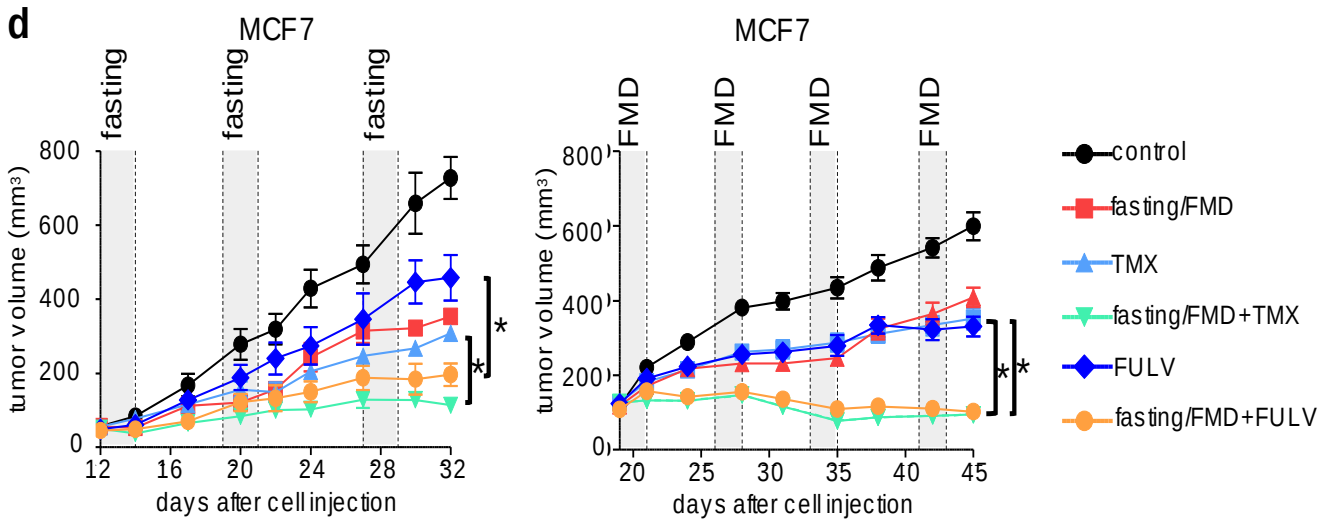
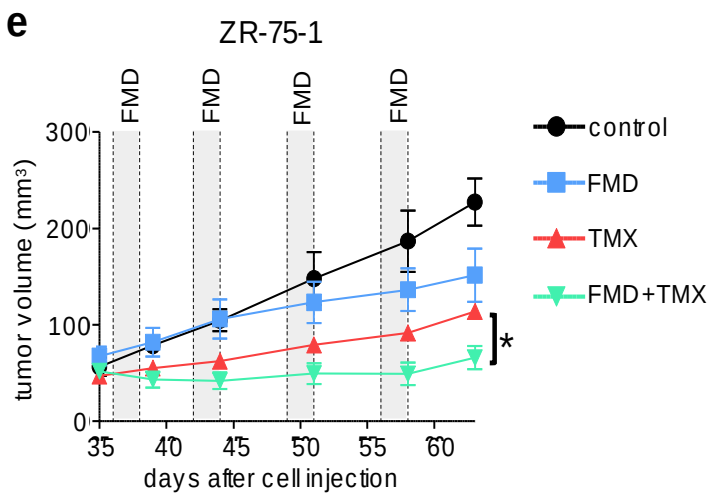
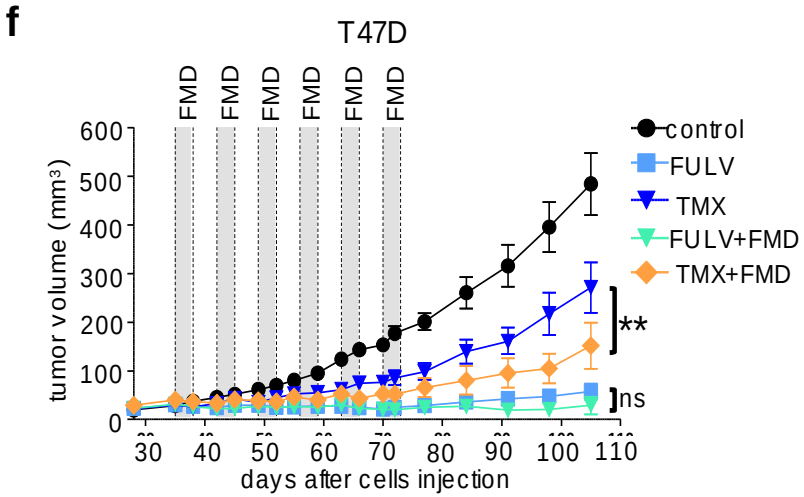
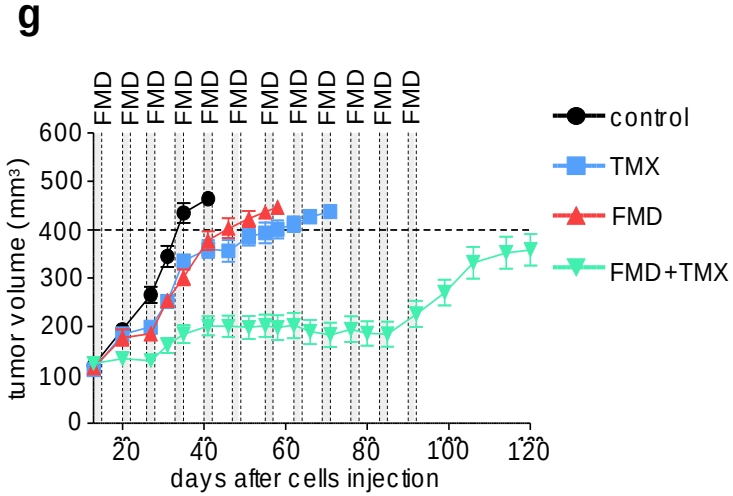


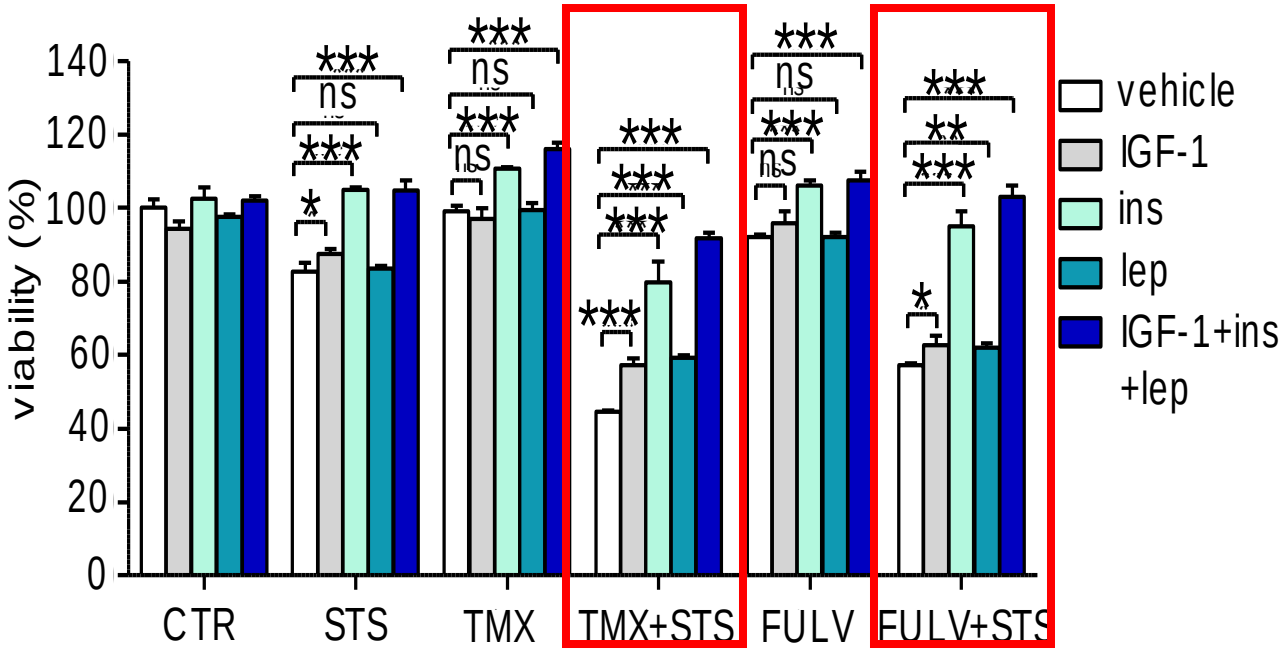




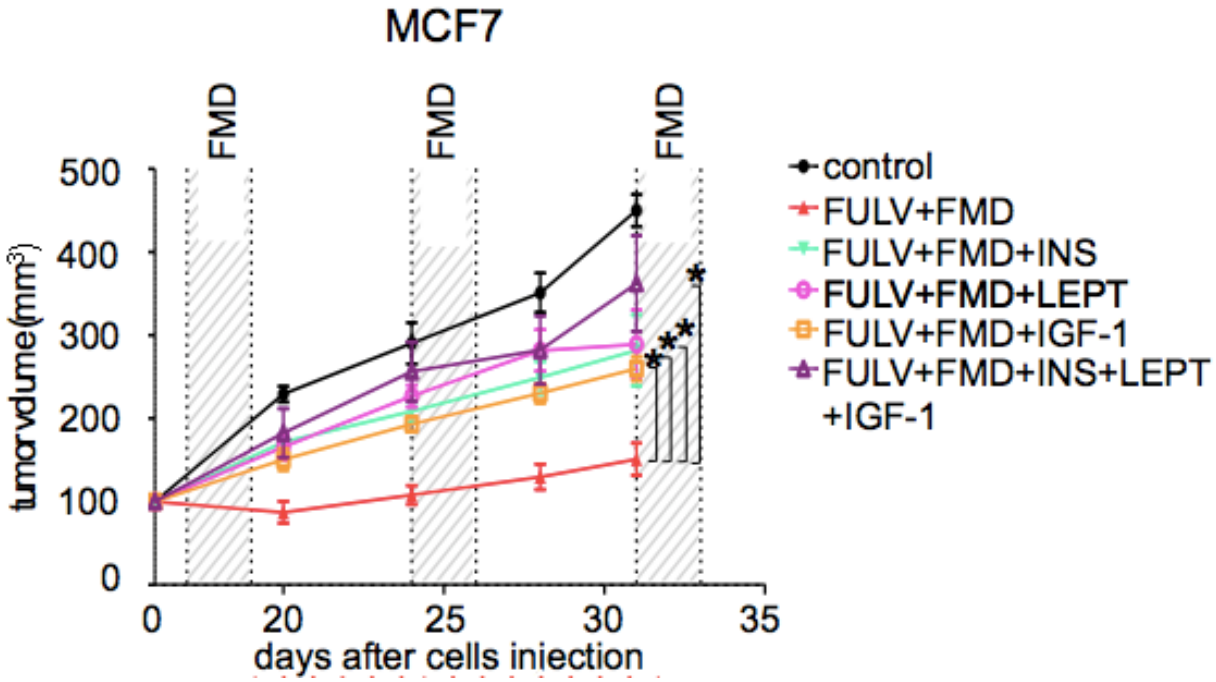


Caffa I, Spagnolo V et al. Nature 2020

Fasting/FMD-induced reduction of blood insulin, leptin and IGF-1 concentration is responsible for its antitumor effects



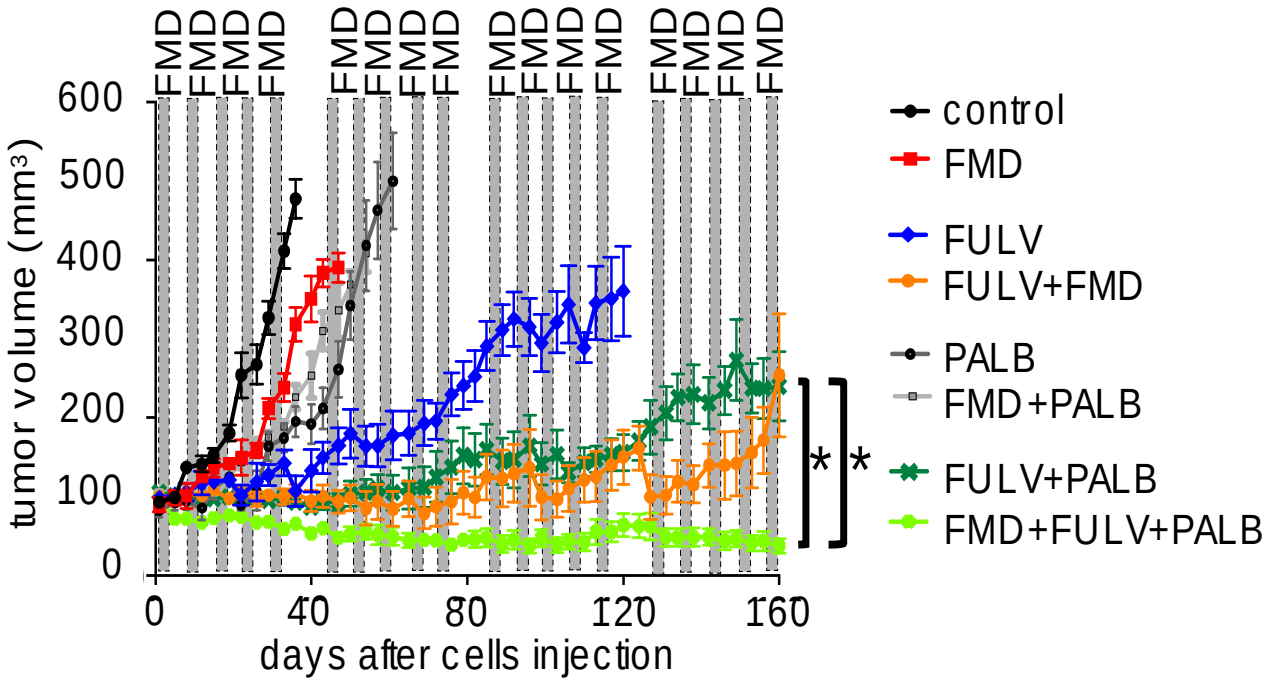
In vitro

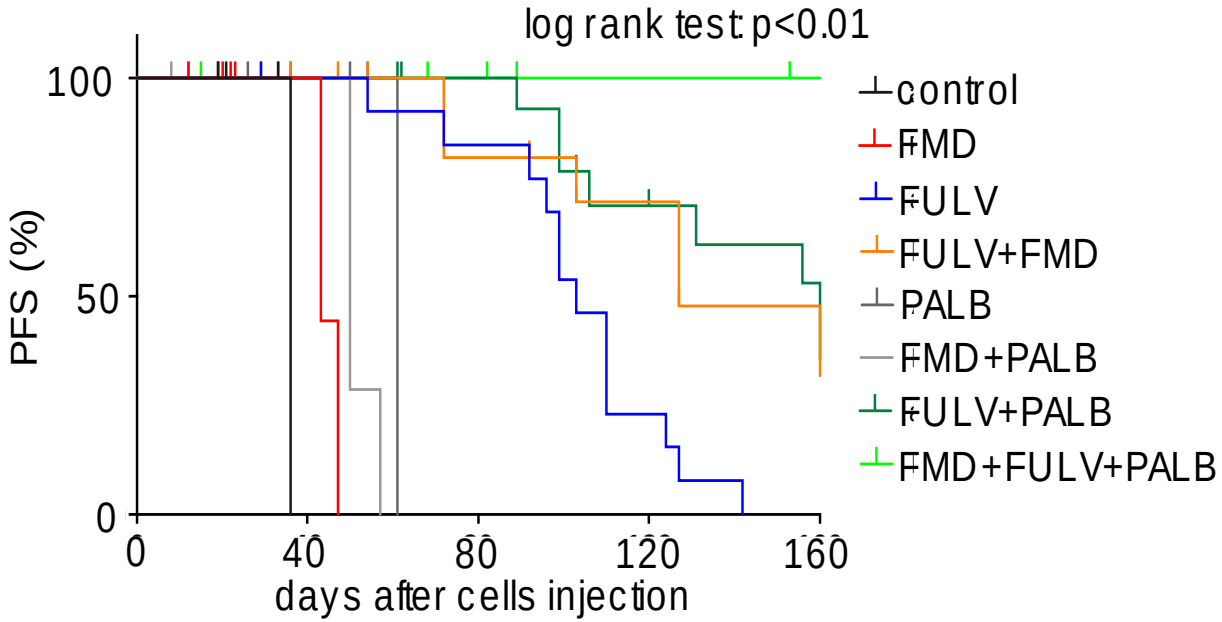


In vivo

Caffa I, Spagnolo V et al. Nature 2020

FMD in combination with Fulvestrant plus Palbociclib results in long term inhibition of *in vivo* tumor growth and in prolongation of animal survival





MCF-7 mouse xenografts

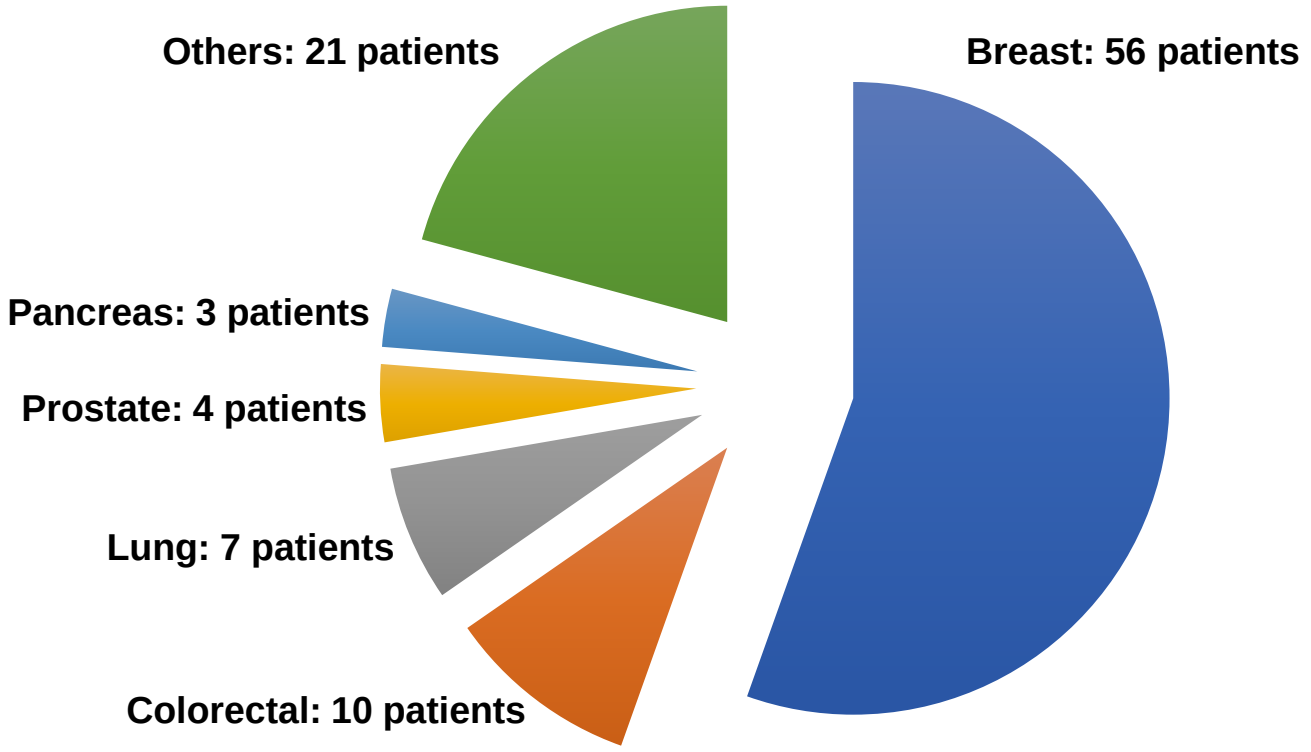
Caffa I, Spagnolo V et al. Nature 2020

Safety, feasibility and metabolic effects of FMD in cancer patients: the INT experience (NCT03340935 trial)

- ~600 Kcal on day 1; ~300 Kcal on days 2-5
- 5-day FMD to be repeated every 21-28 days

Up to a maximum of 8 consecutive FMD cycles

Distribution of cancer types in enrolled patients (n=101)



FMD-related adverse events

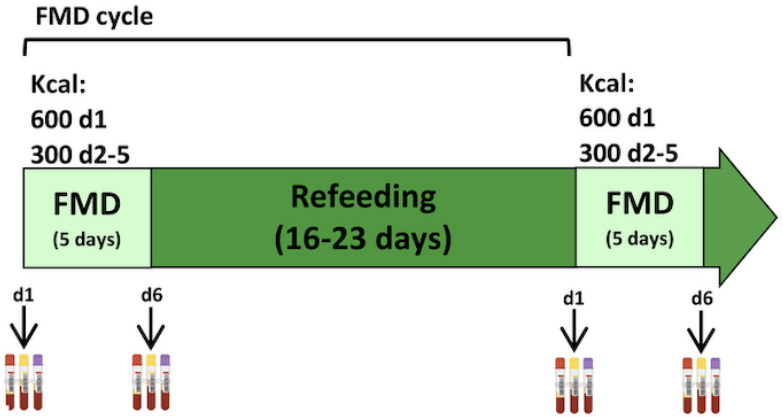
Adverse event	G1 N (%)	G2 N (%)	G3 N (%)	G4 N (%)
Clinical symptoms				
Fatigue	33 (32.7)	54 (53.5)	4 (4.0)	0
Headache	39 (38.6)	1 (1.0)	0	0
Insomnia	14 (13.9)	2 (2.0)	0	0
Somnolence	5 (5.0)	1 (1.0)	0	0
Constipation	9 (8.9)	0	0	0
Muscle Cramps	14 (13.9)	4 (4.0)	0	0
Dizziness	28 (27.7)	8 (7.9)	1 (1.0)	0
Nausea	34 (33.7)	14 (13.9)	1 (1.0)	0
Vomiting	18 (17.8)	3 (3.0)	0	0
Syncope	0	0	1 (1.0)	0
Pre-syncope	5 (5.0)	2 (2.0)	0	0
Tachycardia	5 (5.0)	2 (2.0)	0	0
Epigastric pain	9 (8.9)	4 (4.0)	0	0
Hot flushes	6 (5.9)	0	0	0
Chills	4 (4.0)	0	0	0
Tremor	3 (3.0)	1 (1.0)	0	0
Weight loss	77 (76.2)	0	0	0
Blood tests alterations				
Hypoglycemia	25 (24.8)	17 (16.8)	4 (4.0)	1 (1.0)
AST increased	28 (27.7)	0	1 (1.0)	0
ALT increased	27 (26.7)	2 (2.0)	0	0
Uricemia increased	13 (12.9)	0	0	0
Total cholesterol increased	18 (17.8)	1 (1.0)	0	0
Hypertriglyceridemia	13 (12.9)	2 (2.0)	0	0
Creatinine increased	5 (5.0)	0	0	0
Total FMD-related adverse events	100 (99.0)	70 (69.3)	12 (11.9)	1 (1.0)
Total rate of G3/4 FMD-related adverse events: 12.9%; 90% CIs: 7.8-19.7%				

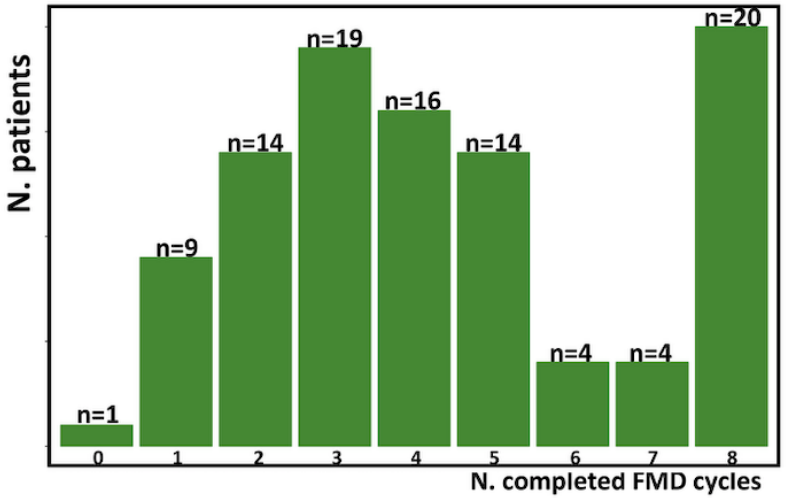
Abbreviations: N, number; G, Grade

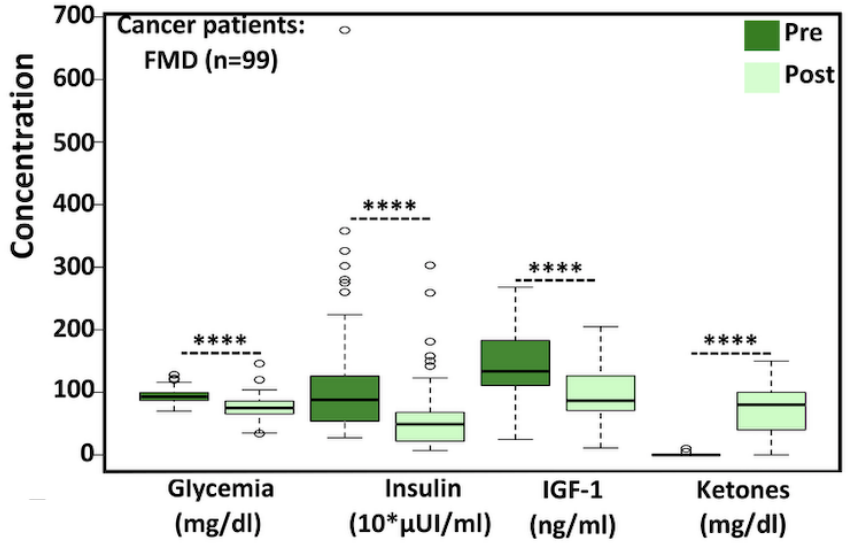
Incidence of G3/G4 FMD-related AEs: 12.9%, lower than the pre-specified 20% threshold

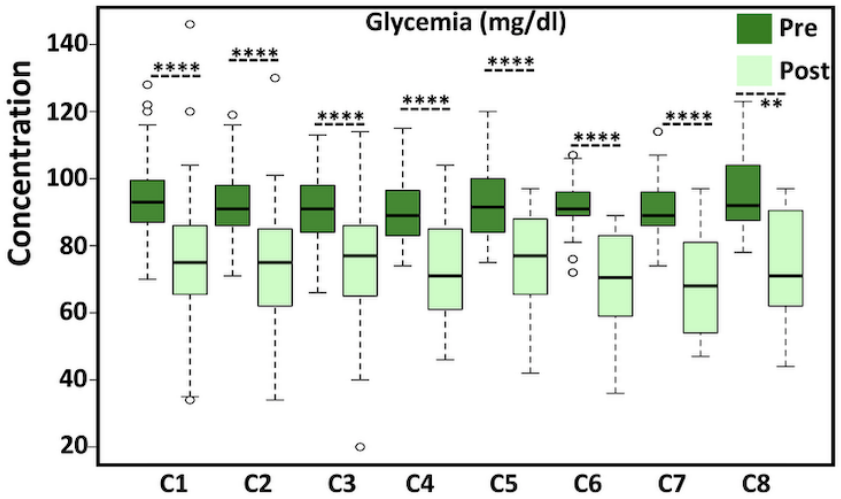
Vernieri C et al. Cancer Discov 2022

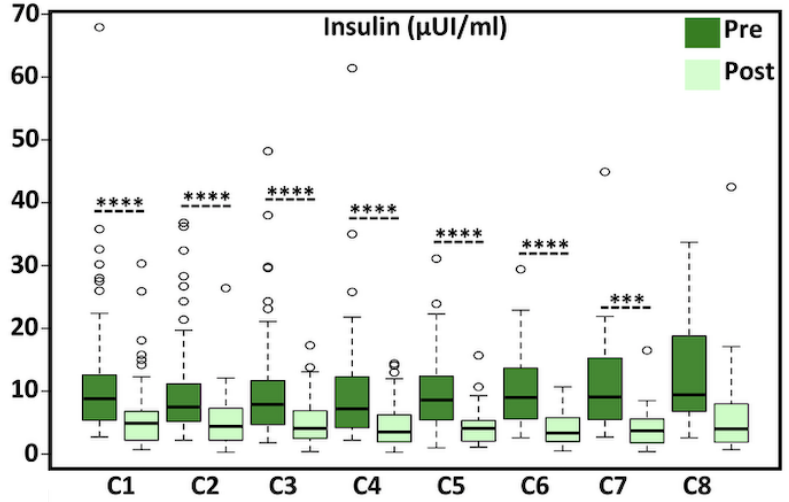
FMD rephapes systemic metabolism in a way that reproduces the metabolic effects of fasting/FMD in tumor-bearing mice

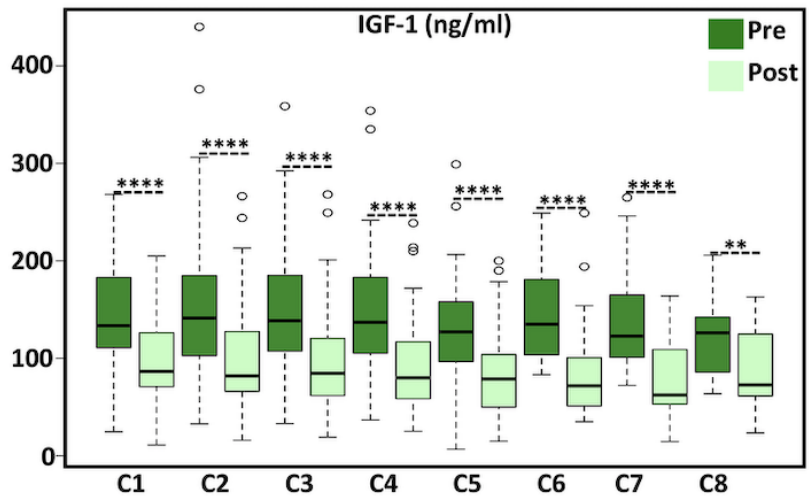












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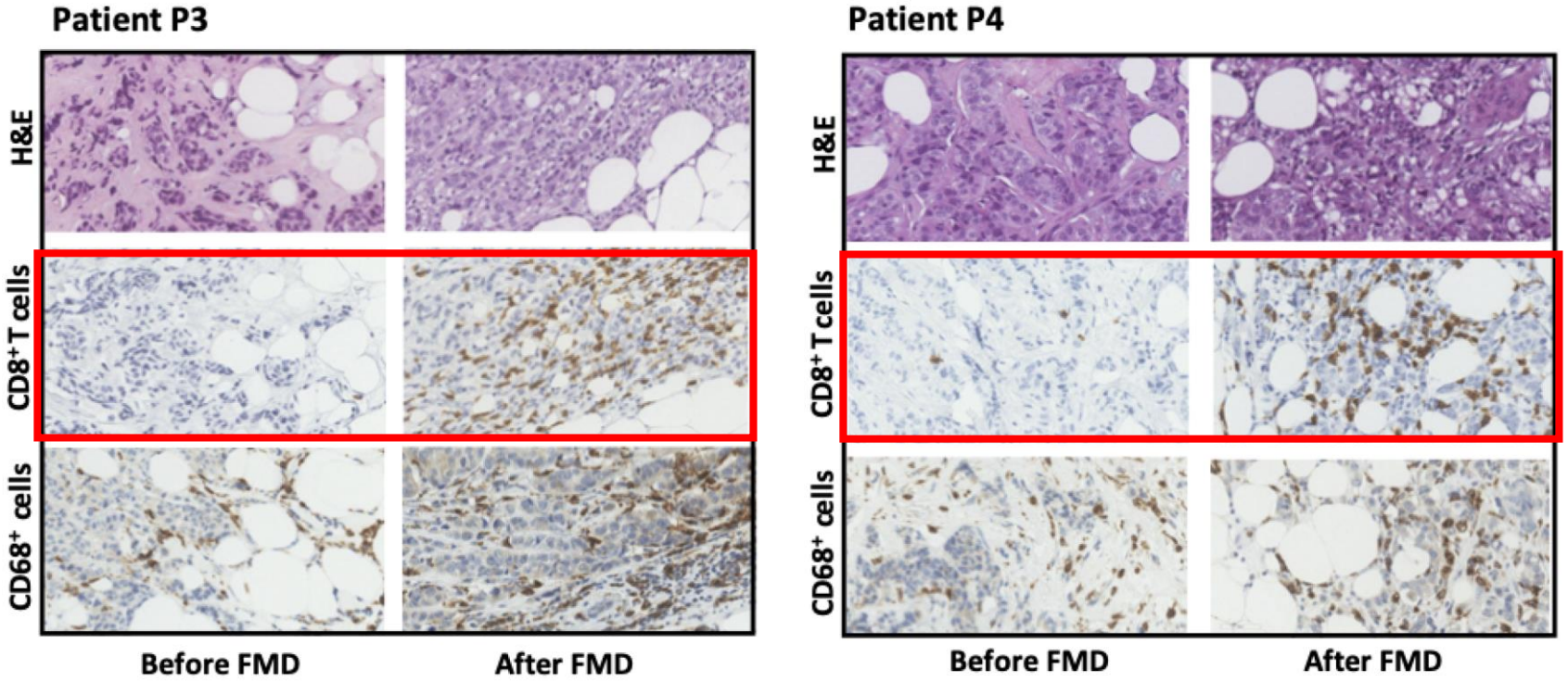
Cyclic FMD stimulates tumor infiltration by CD8$^+$ T cells and reduces the CD8/CD68 ration in patients with limited-stage breast cancer



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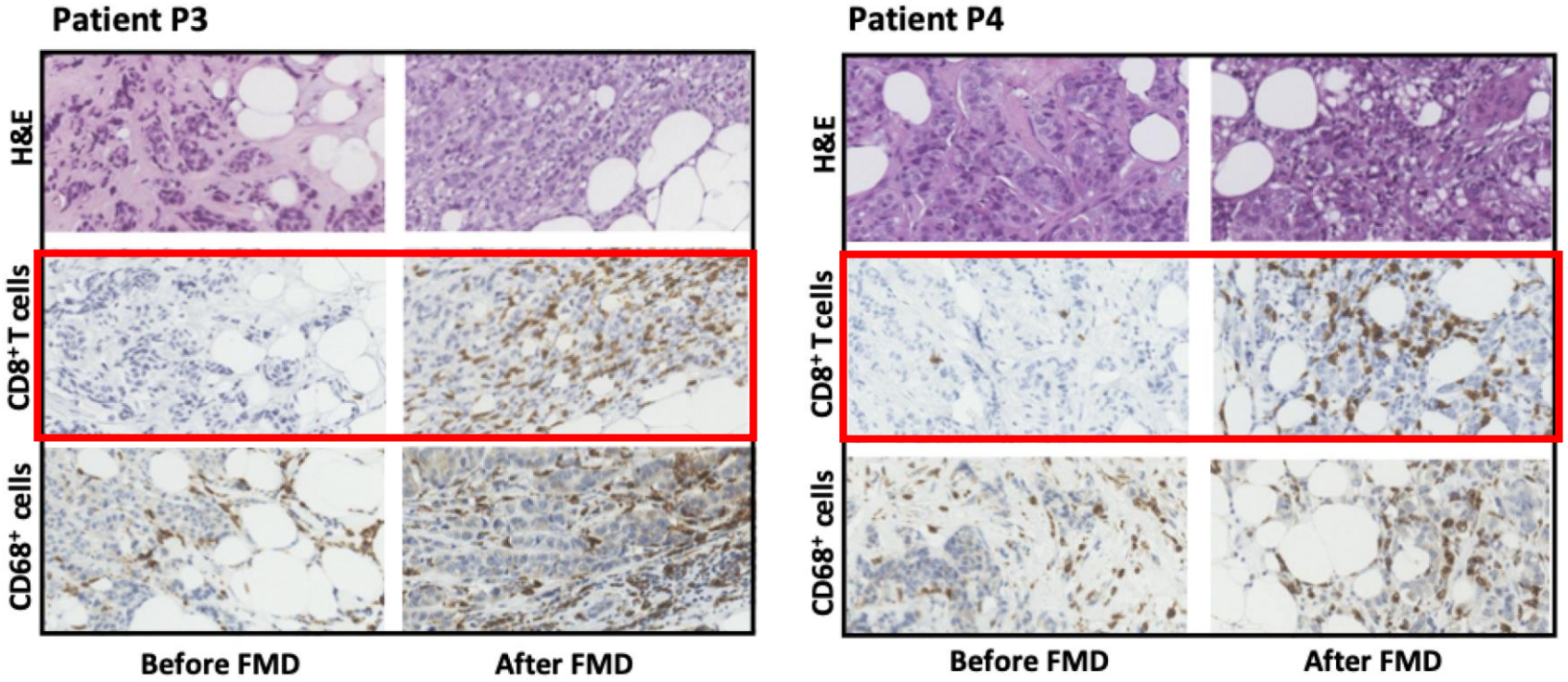
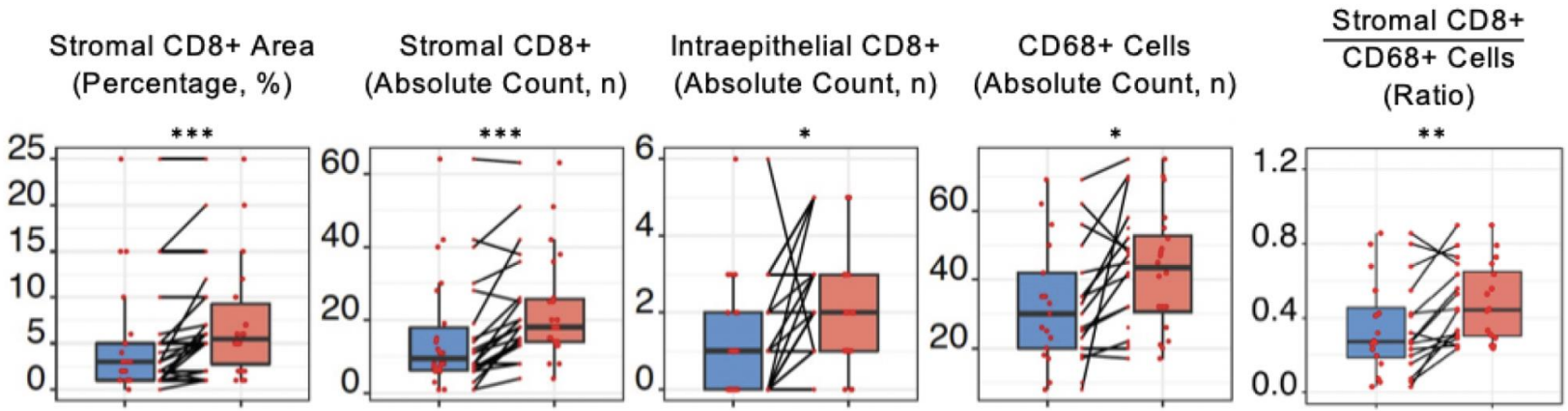
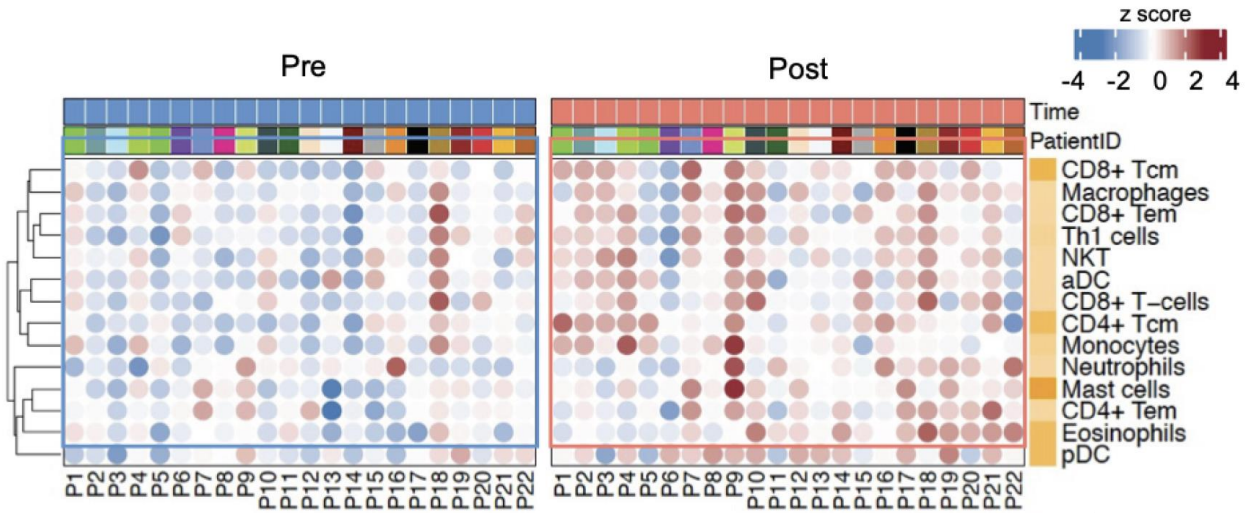




Vernieri C et al. Cancer Discov 2022

Cyclic FMD globally reshapes the intratumor immune contexture

Estimates of tumor-infiltrating immune cell subsets by deconvolution analysis of RNA-sea data



N=22 patients

Vernieri C et al. Cancer Discov 2022

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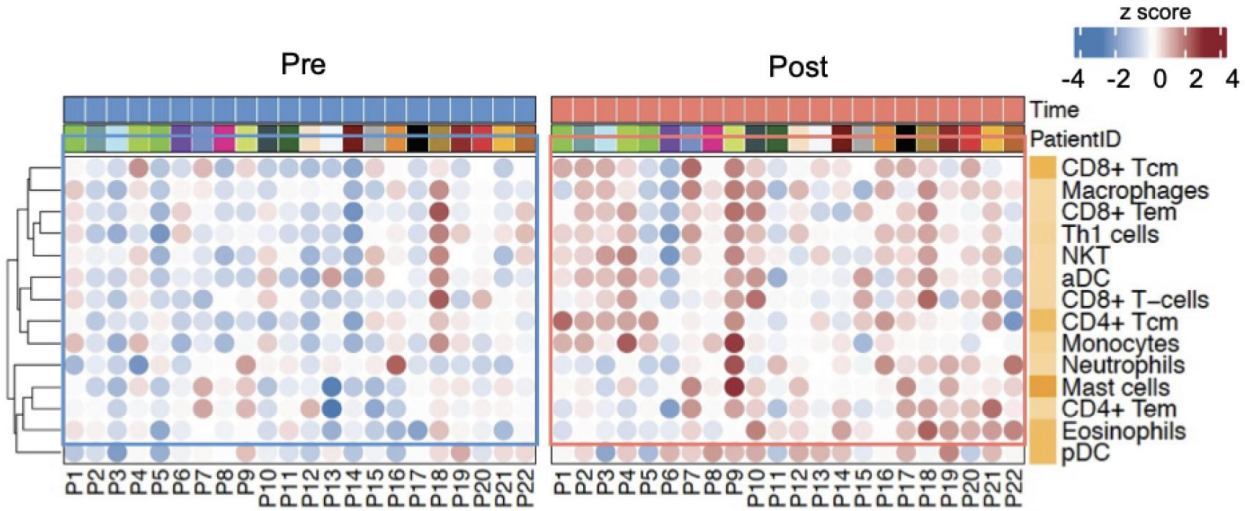
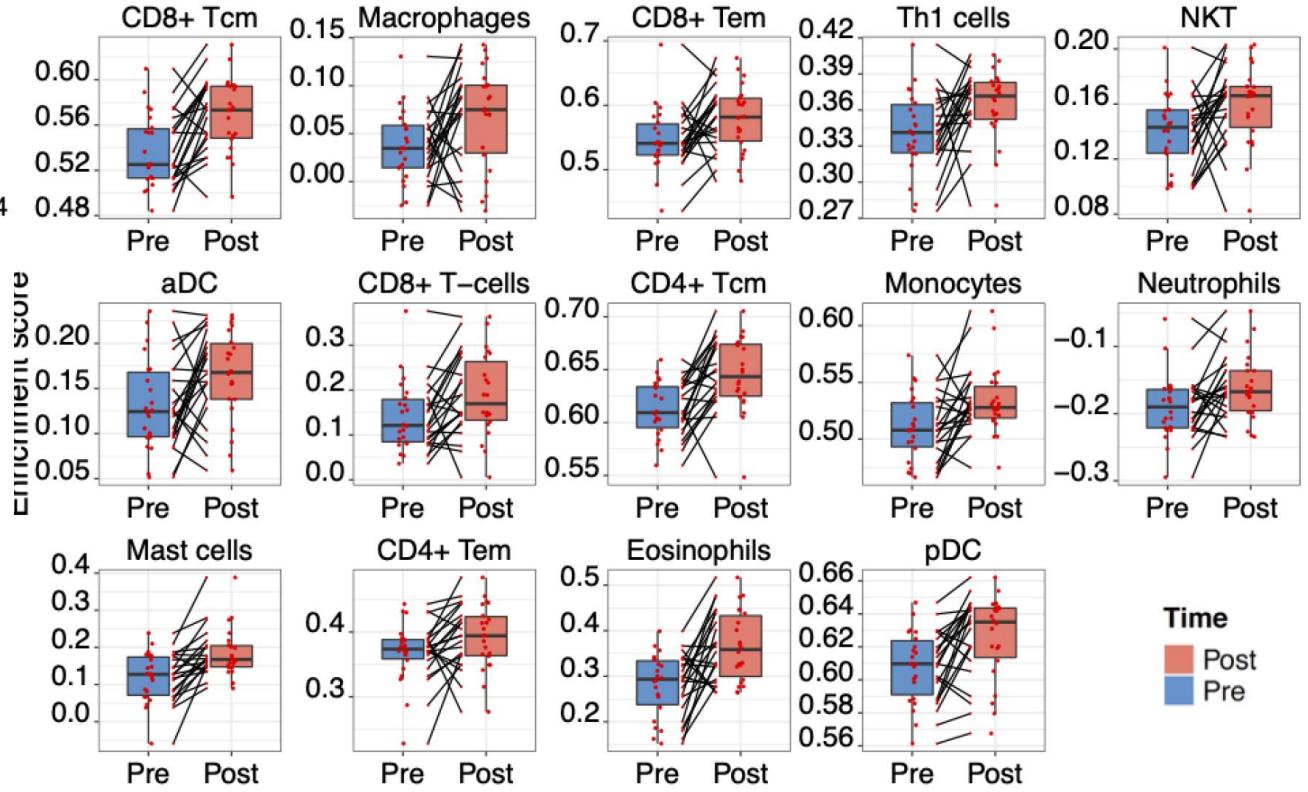


Estimated tumor-infiltrating immune cell populations before and after the FMD

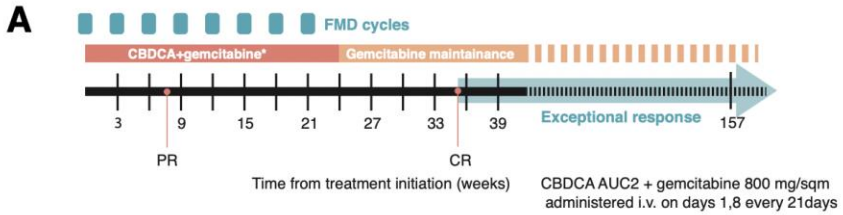


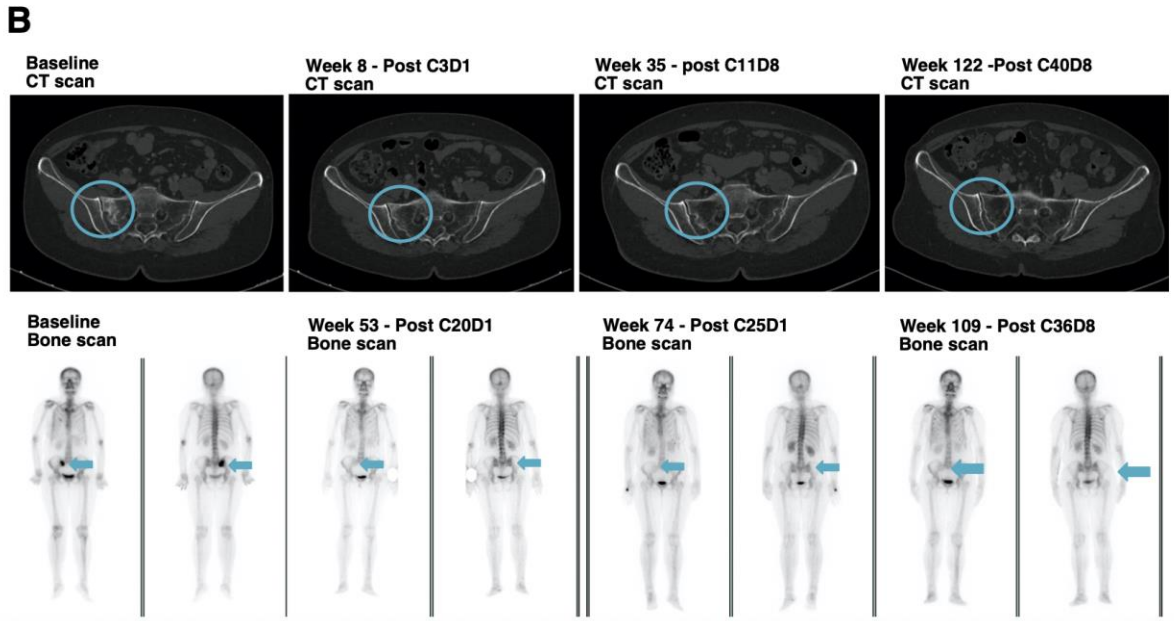
N=22 patients

Vernieri C et al. Cancer Discov 2022

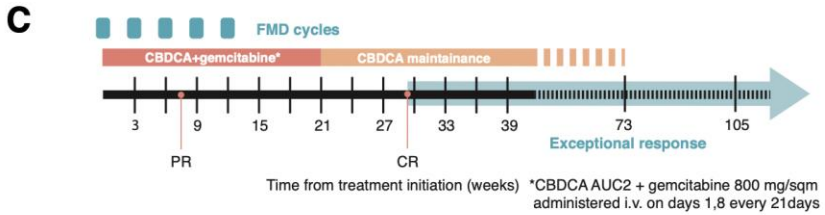
Two advanced TNBC patients among exceptional responders receiving cyclic FMD plus standard platinum-based chemotherapy

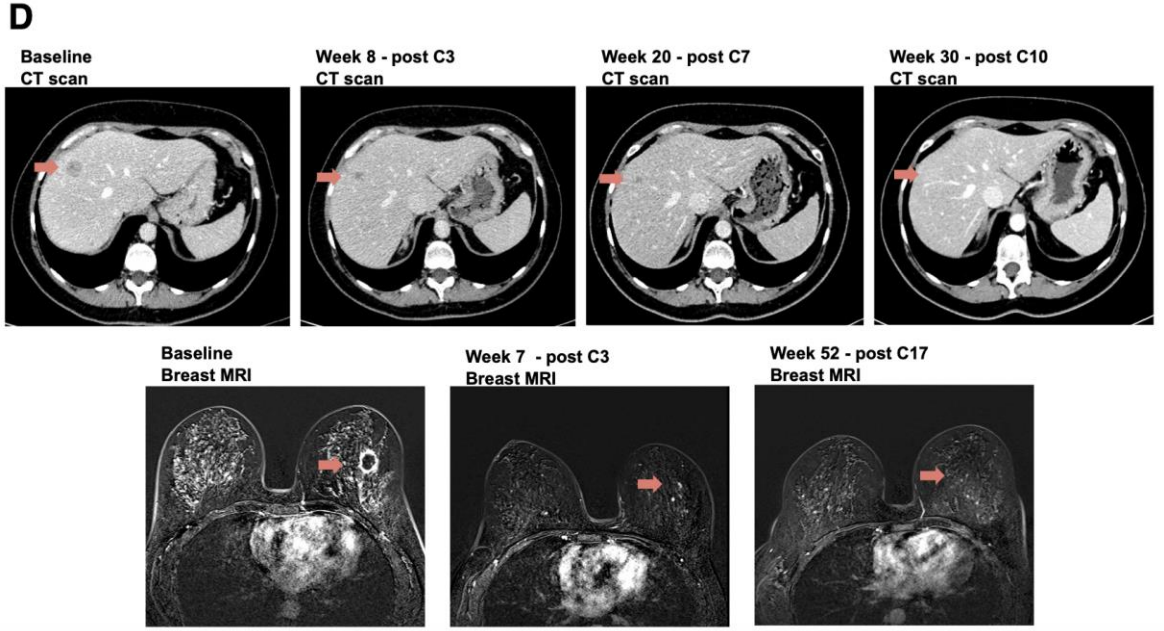
Patient n.1: TNBC





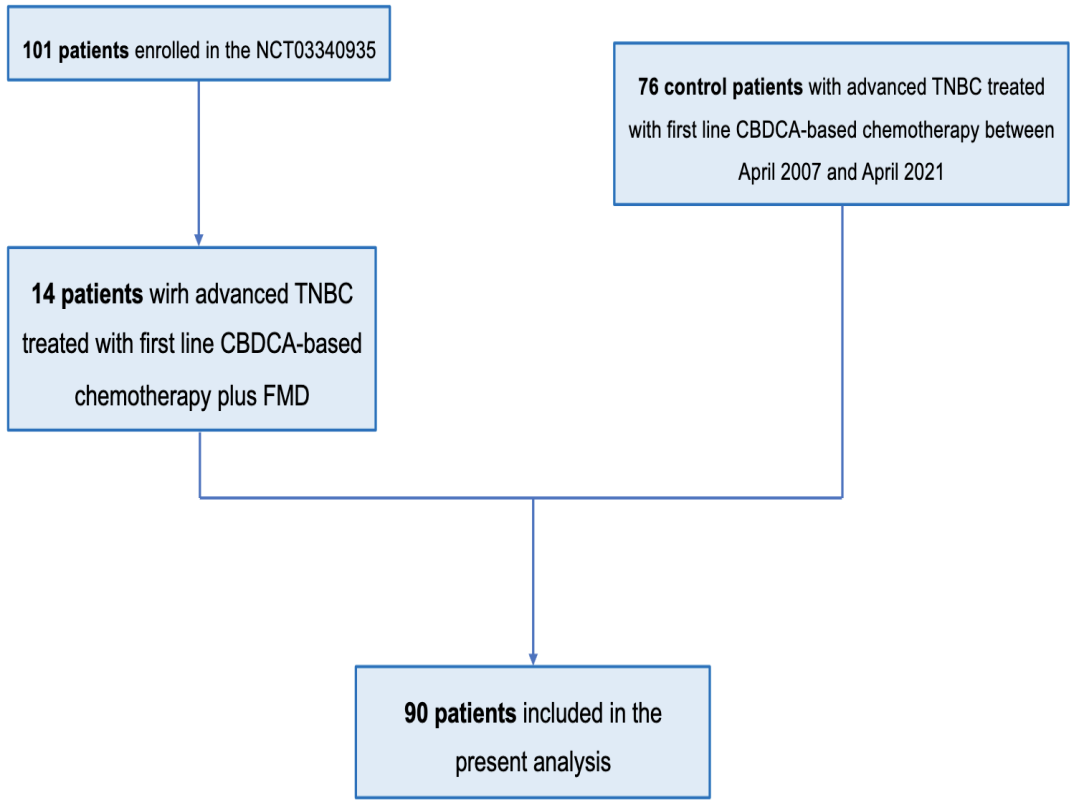
Patient n.2: TNBC

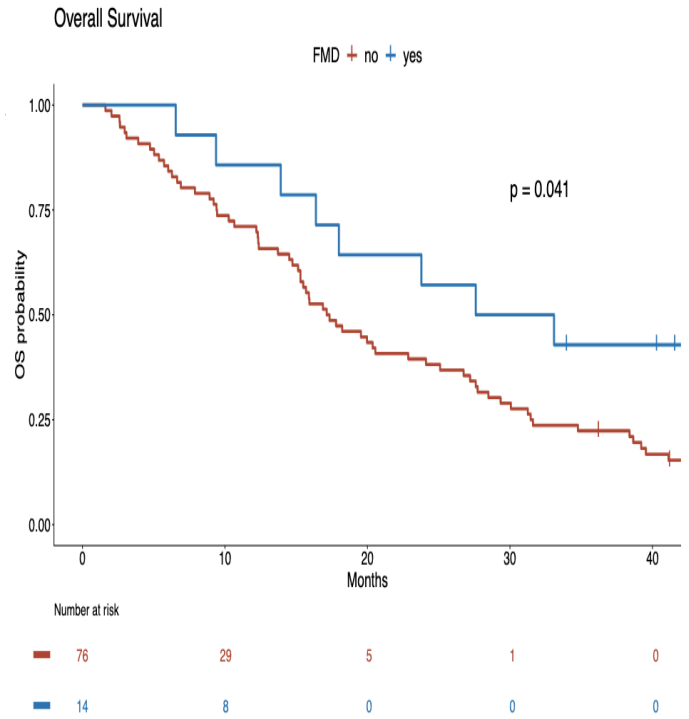


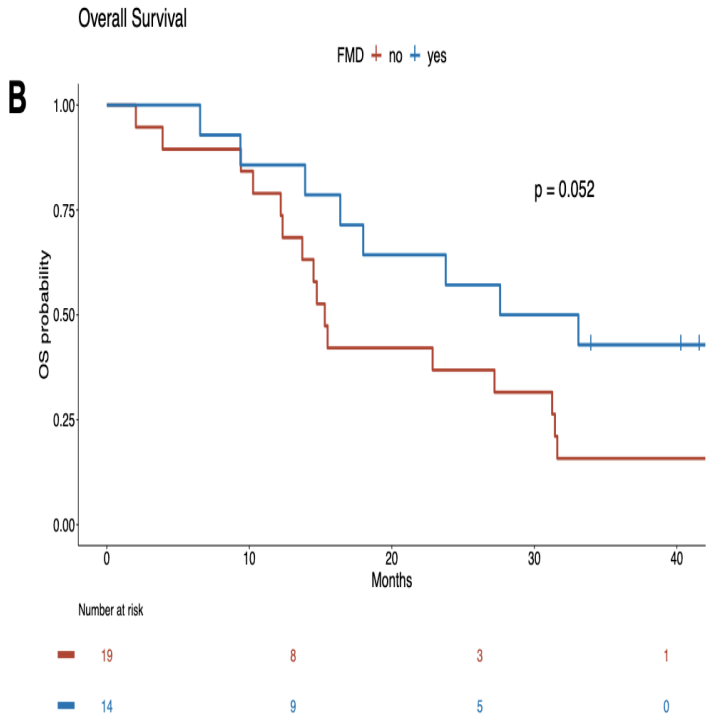


Ligorio F et al. Eur J Cancer 2022

Combining cyclic FMD with first-line platinum chemotherapy is associated with better OS when compared to platinum chemotherapy alone in aTNBC patients

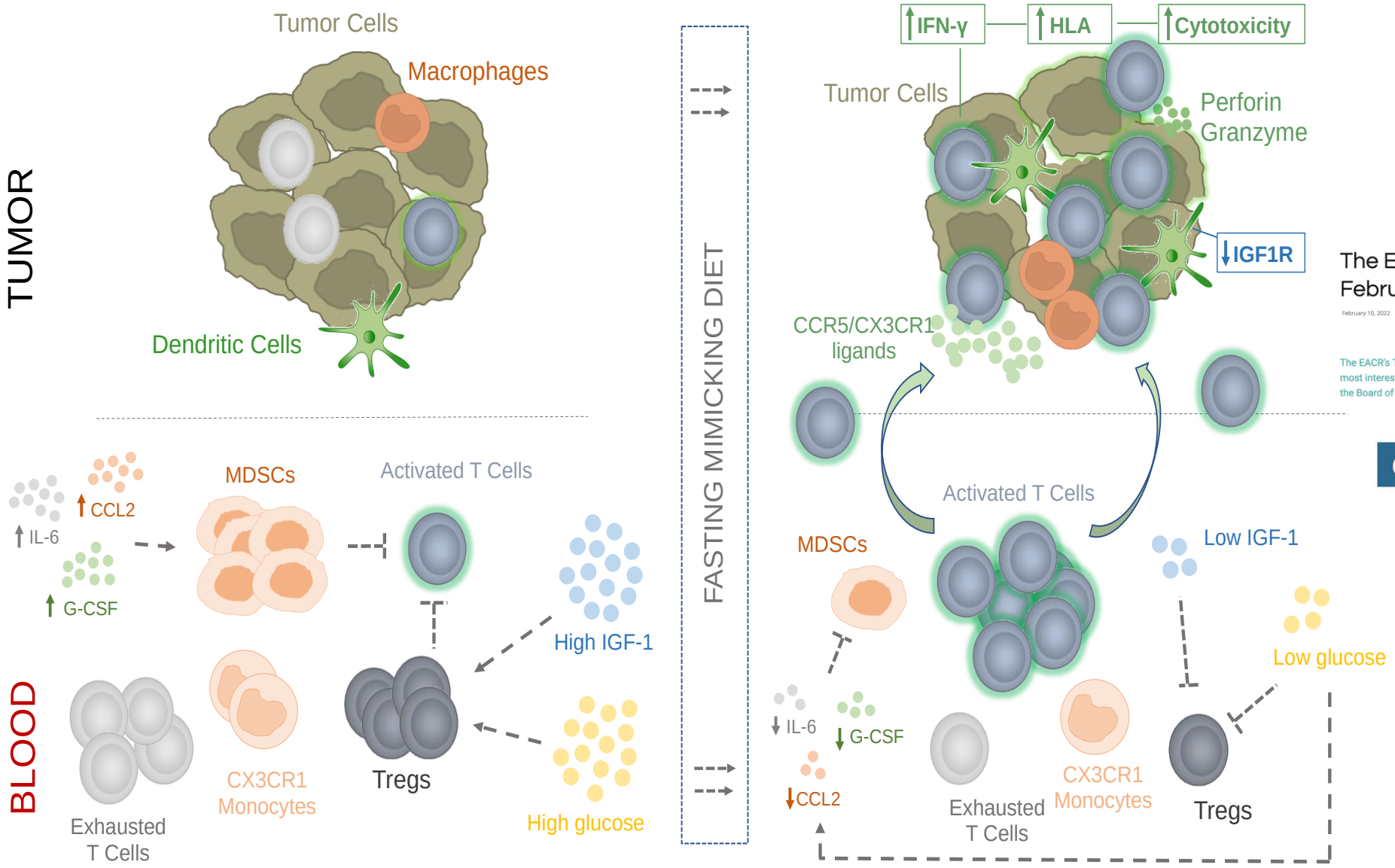






Ligorio F et al. Int J Cancer 2024

Cyclic FMD reduces immunosuppressive cell subsets, while boosting systemic and intratumor immunity



The EACR's Top 10 Cancer Research Publications: February 2022

February 10, 2022

The EACR's Top 10 Cancer Research Publications is a regular summary of the most interesting and impactful recent papers in cancer research. It is curated by the Board of the European Association for Cancer Research (EACR).

EACR European Association for Cancer Research

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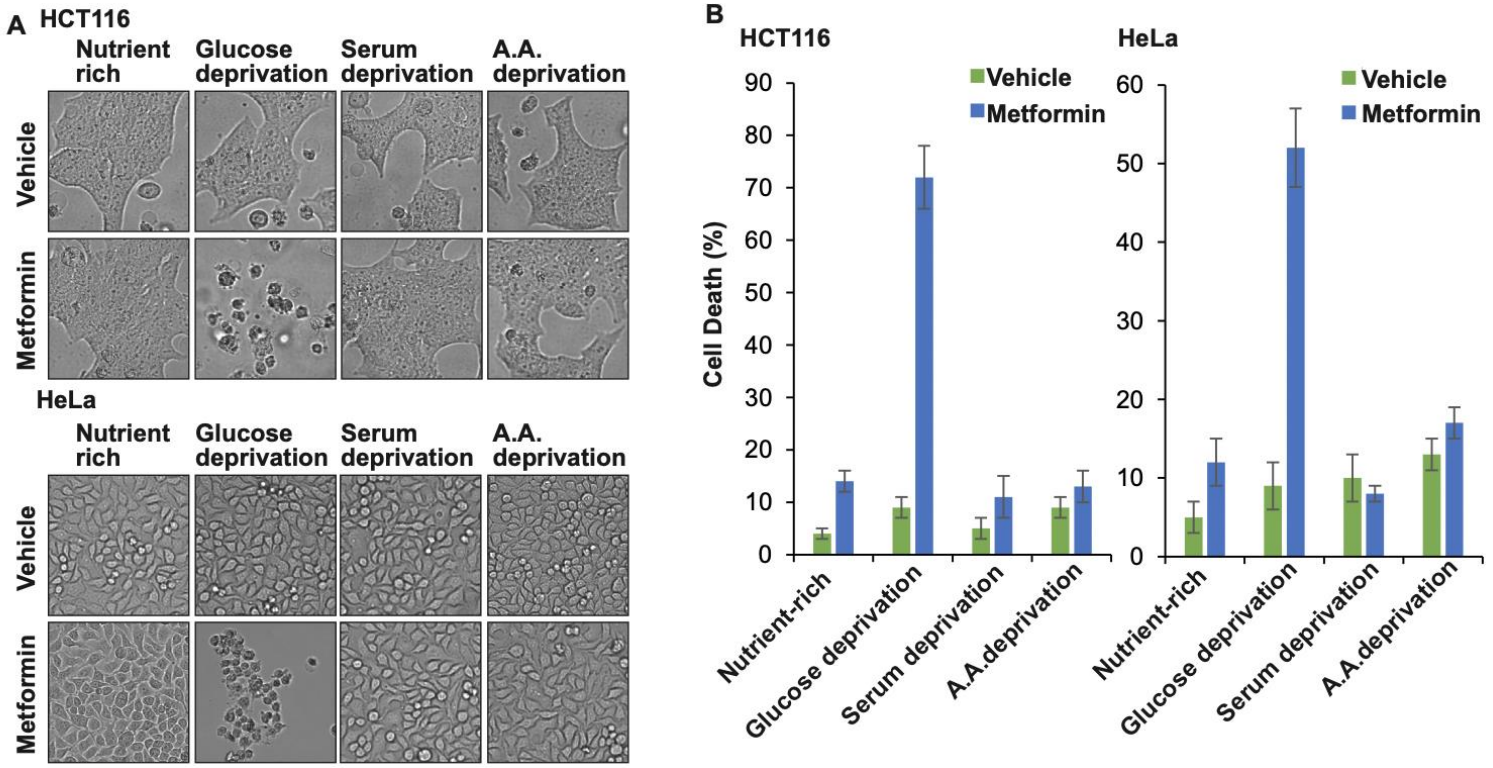
6 **Fasting-Mimicking Diet Is Safe and Reshapes Metabolism and Antitumor Immunity in Patients with Cancer**

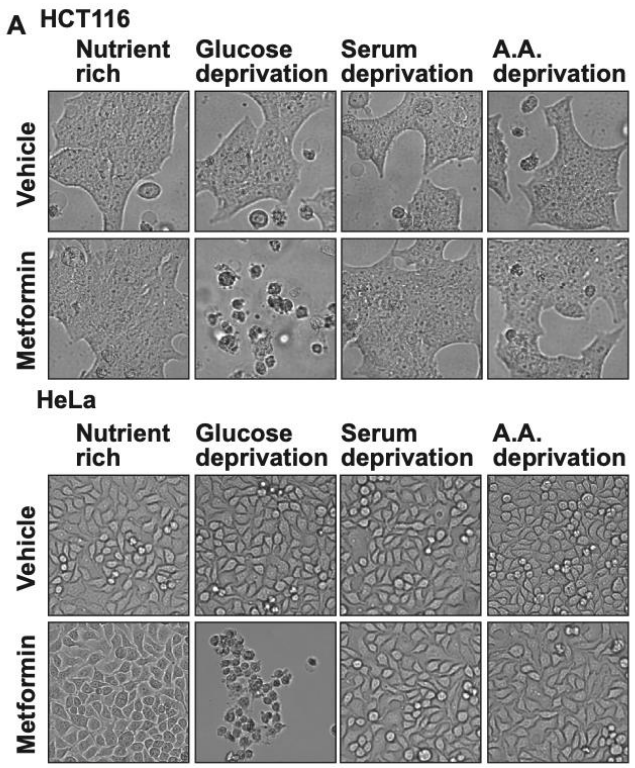
Vernieri C et al. Cancer Discov 2022

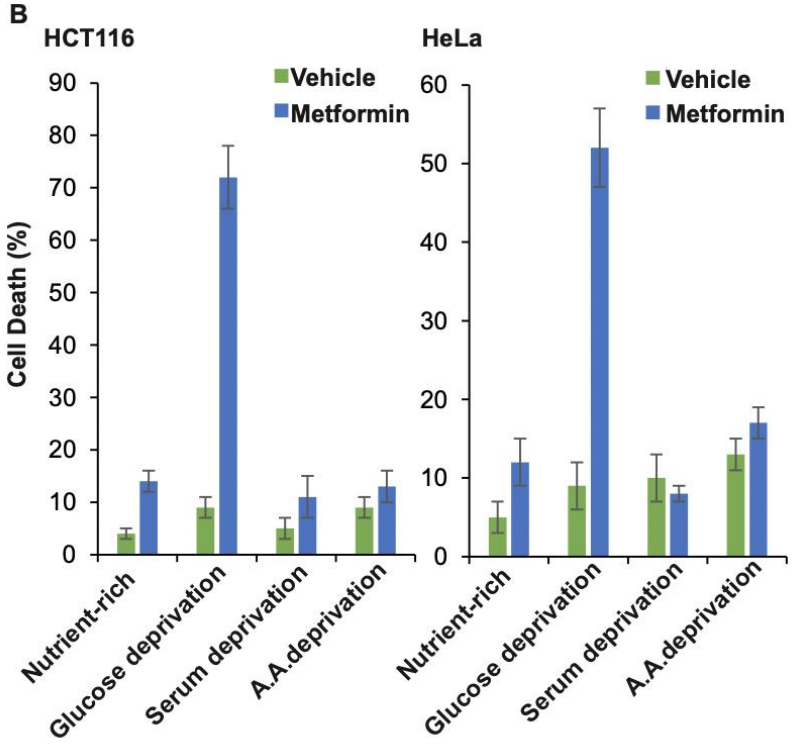
Glucose deprivation synergizes with the OXPHOS inhibitor metformin by causing acute metabolic stress

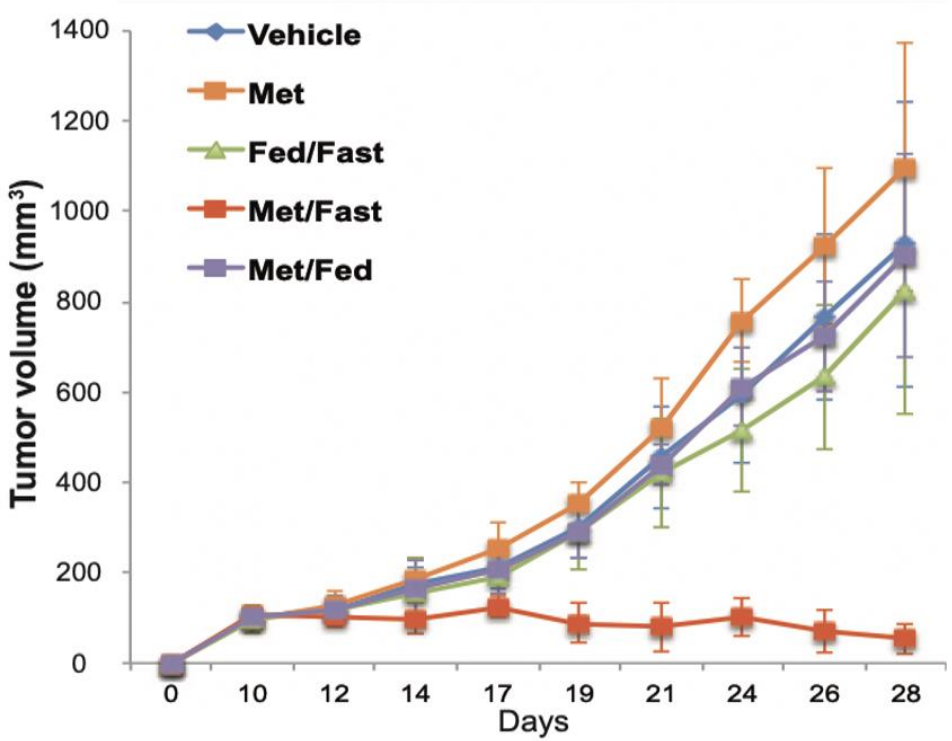


Elgendy M et al. Cancer Cell 2019

Glucose deprivation synergizes with the OXPHOS inhibitor metformin by causing acute metabolic stress

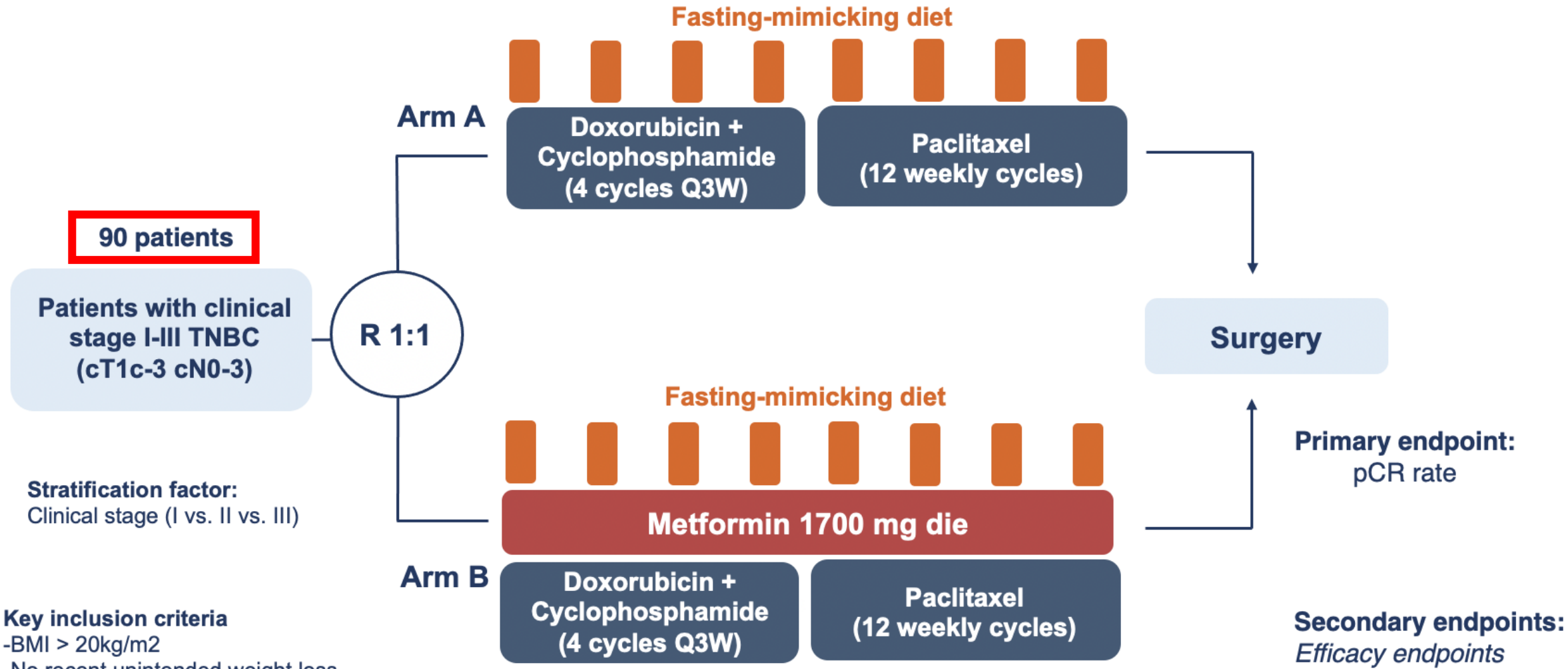




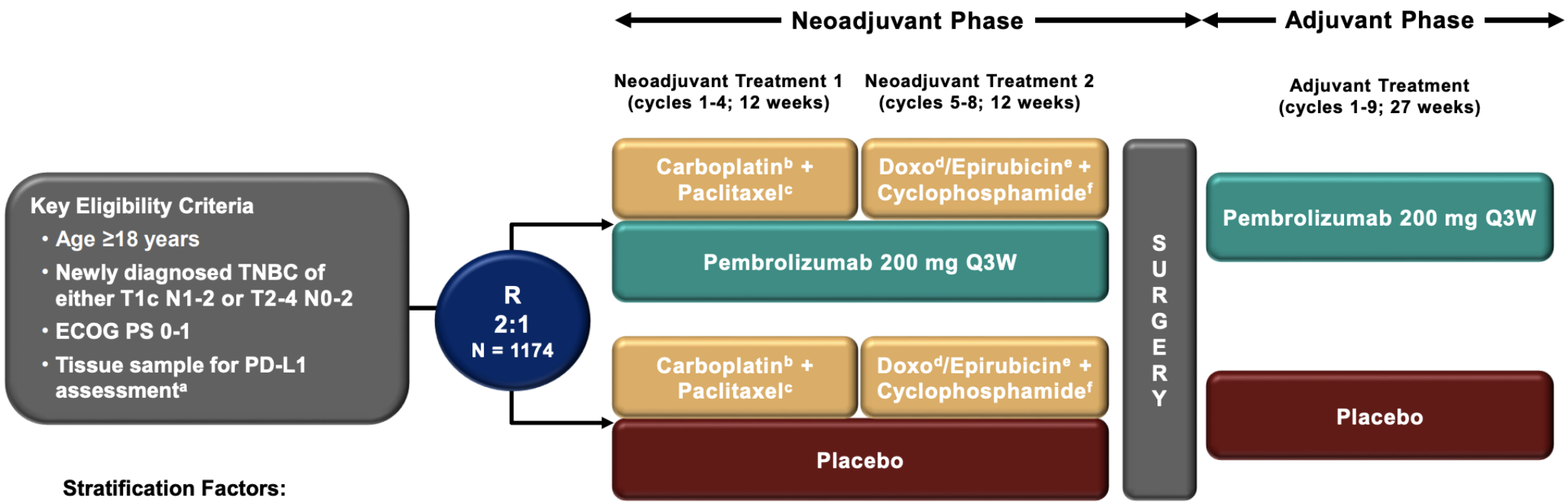


Elgendy M et al. Cancer Cell 2019

FMD plus/minus metformin in early-stage TNBC: the phase II trial BREAKFAST (NCT004248998)



KEYNOTE-522 Study Design (NCT03036488)



Stratification Factors:

- Nodal status (+ vs -)
- Tumor size (T1/T2 vs T3/T4)
- Carboplatin schedule (QW vs Q3W)

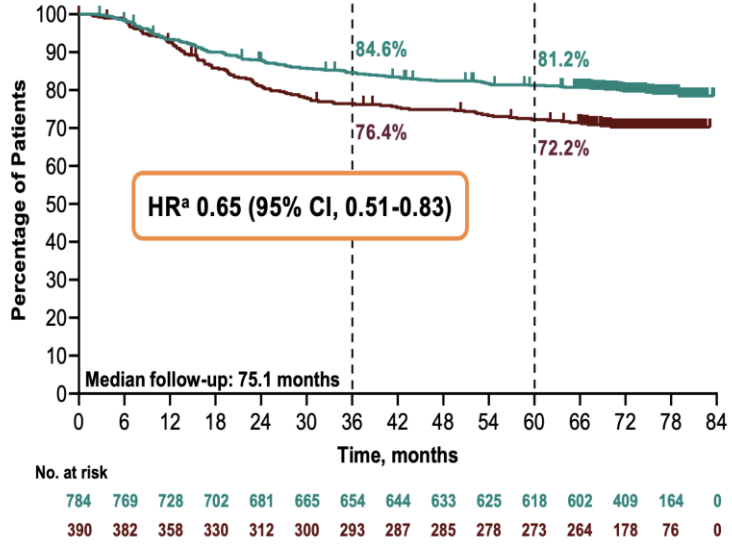
Neoadjuvant phase: starts from the first neoadjuvant treatment and ends after definitive surgery (post-treatment included)

Adjuvant phase: starts from the first adjuvant treatment and includes radiation therapy as indicated (post-treatment included)

[a]Must consist of at least 2 separate tumor cores from the primary tumor. [b]Carboplatin dose was AUC 5 Q3W or AUC 1.5 QW. [c]Paclitaxel dose was 80 mg/m^2 QW. [d]Doxorubicin dose was 60 mg/m^2 Q3W. [e]Epirubicin dose was 90 mg/m^2 Q3W. [f]Cyclophosphamide dose was 600 mg/m^2 Q3W.

Chemoimmunotherapy improves EFS and OS when compared to chemotherapy alone in early-stage TNBC patients

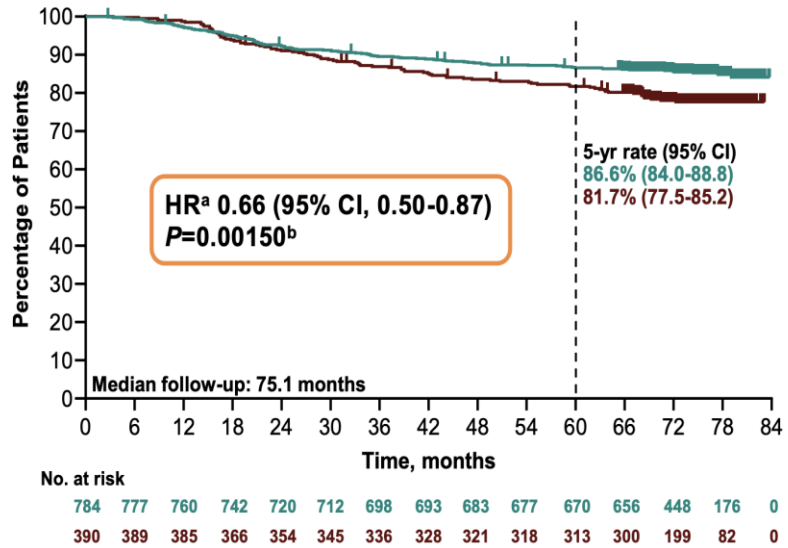
Updated Event-Free Survival



	Pts w/ Event
Pembro + Chemo/Pembro	20.3%
Placebo + Chemo/Placebo	29.2%

[a]Hazard ratio (CI) analyzed based on a Cox regression model with treatment as a covariate stratified by the randomization stratification factors. Data cutoff date: March 22, 2024.

Key Secondary Endpoint: Overall Survival

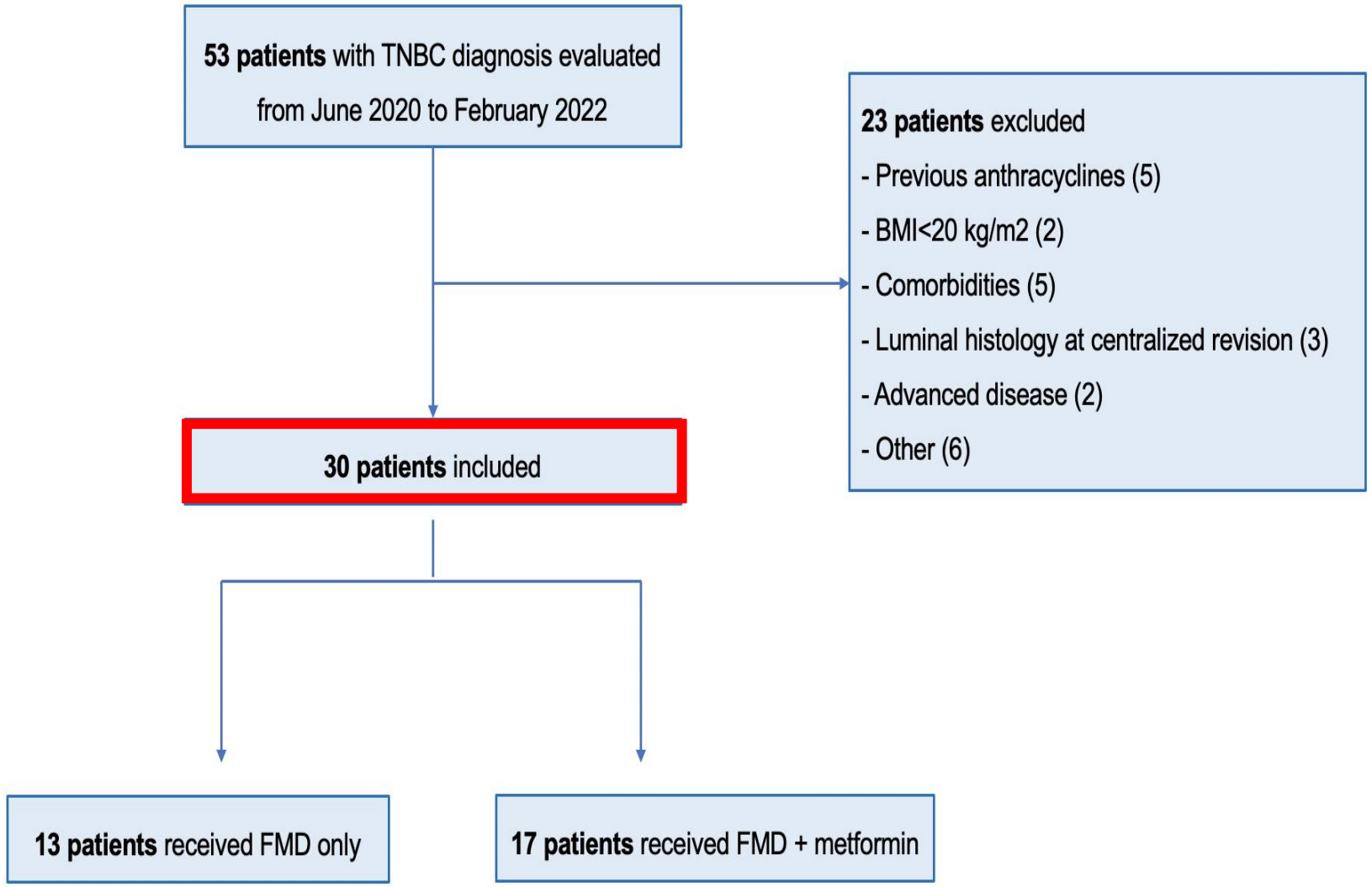


	Pts w/ Event
Pembro + Chemo/Pembro	14.7%
Placebo + Chemo/Placebo	21.8%

[a]The unstratified piecewise HR was 0.87 (95% CI, 0.57-1.32) before the 2-year follow-up and 0.51 (95% CI, 0.35-0.75) afterwards. The weighted average HR with weights of number of events before and after 2-year follow-up was 0.66. With 200 events (67.3% information fraction), the observed *P*-value crossed the prespecified nominal boundary of 0.00503 (1-sided) at this interim analysis. Data cutoff date: March 22, 2024.

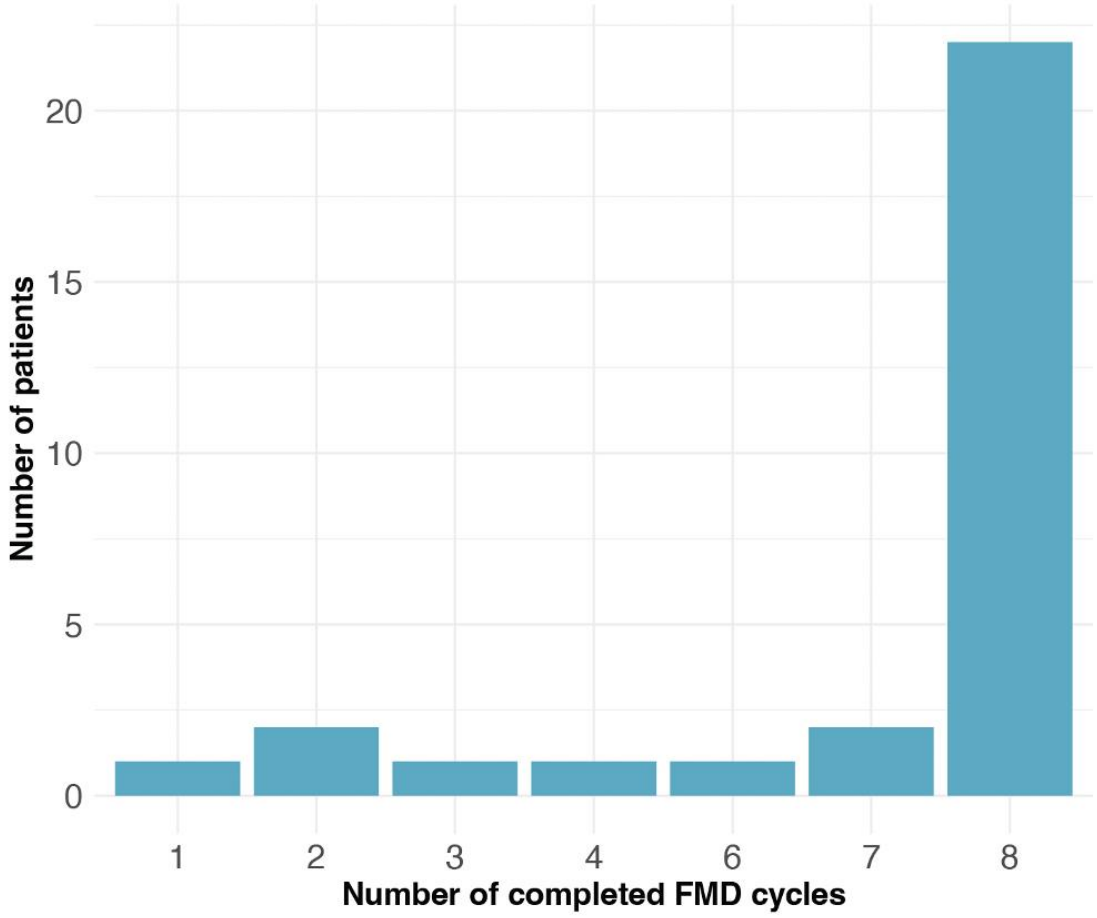
Schmid P. ESMO meeting 2024

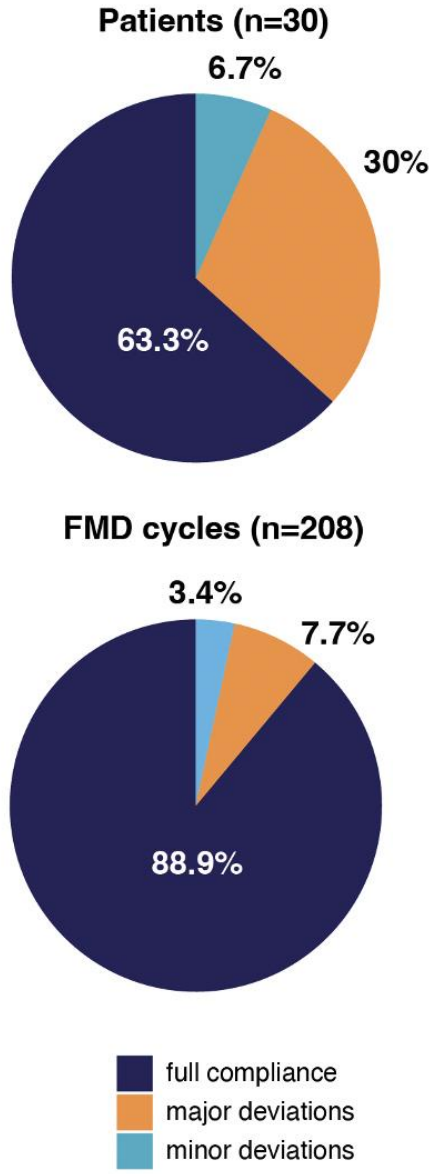
Study flow chart



Ligorio F et al. Cell Metabolism 2025

Patient compliance to FMD was excellent

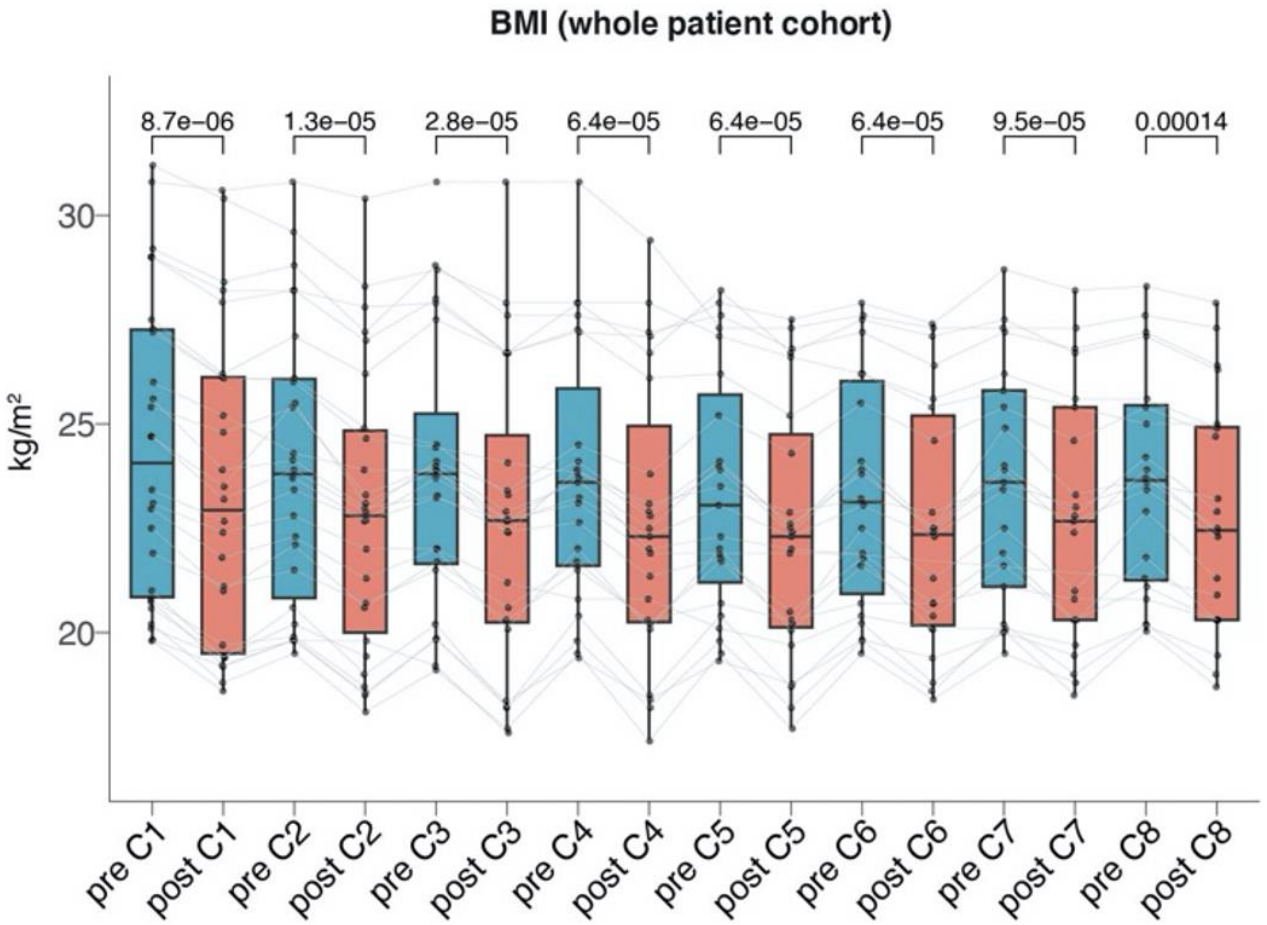


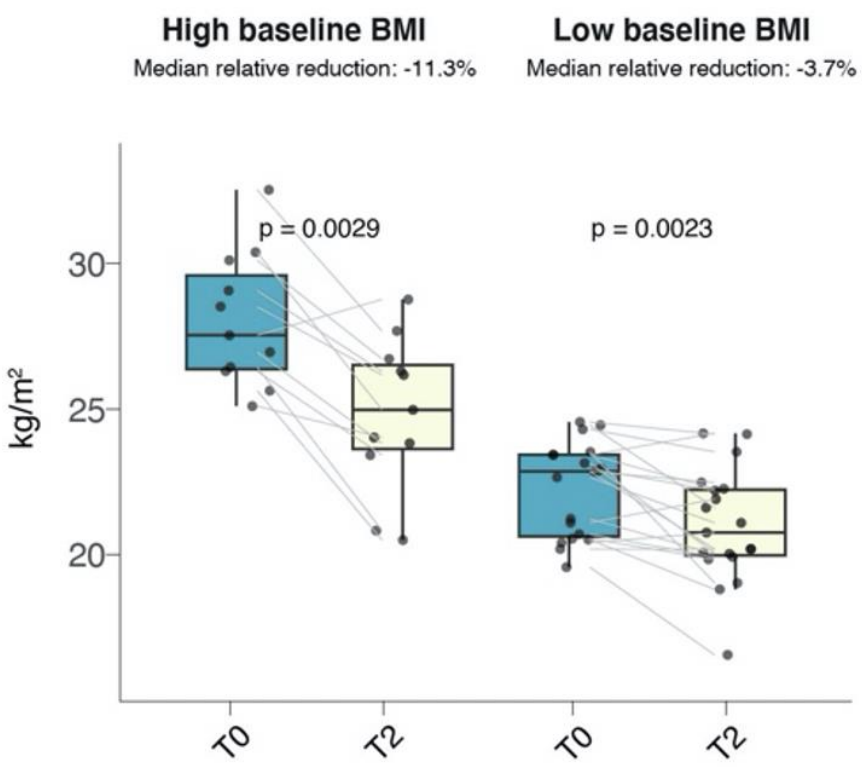


Vernieri C. SABCS 2023

Ligorio F et al. Cell Metabolism 2025

Overweight/obese patients undergo the highest BMI reduction during chemotherapy plus FMD

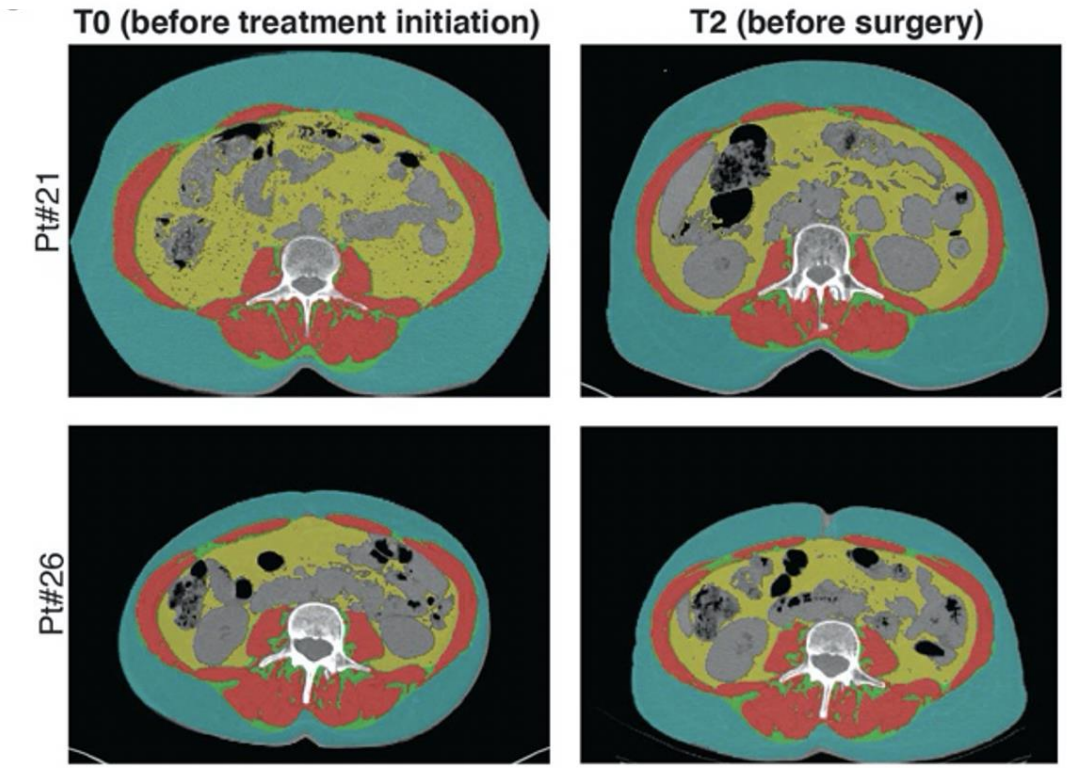


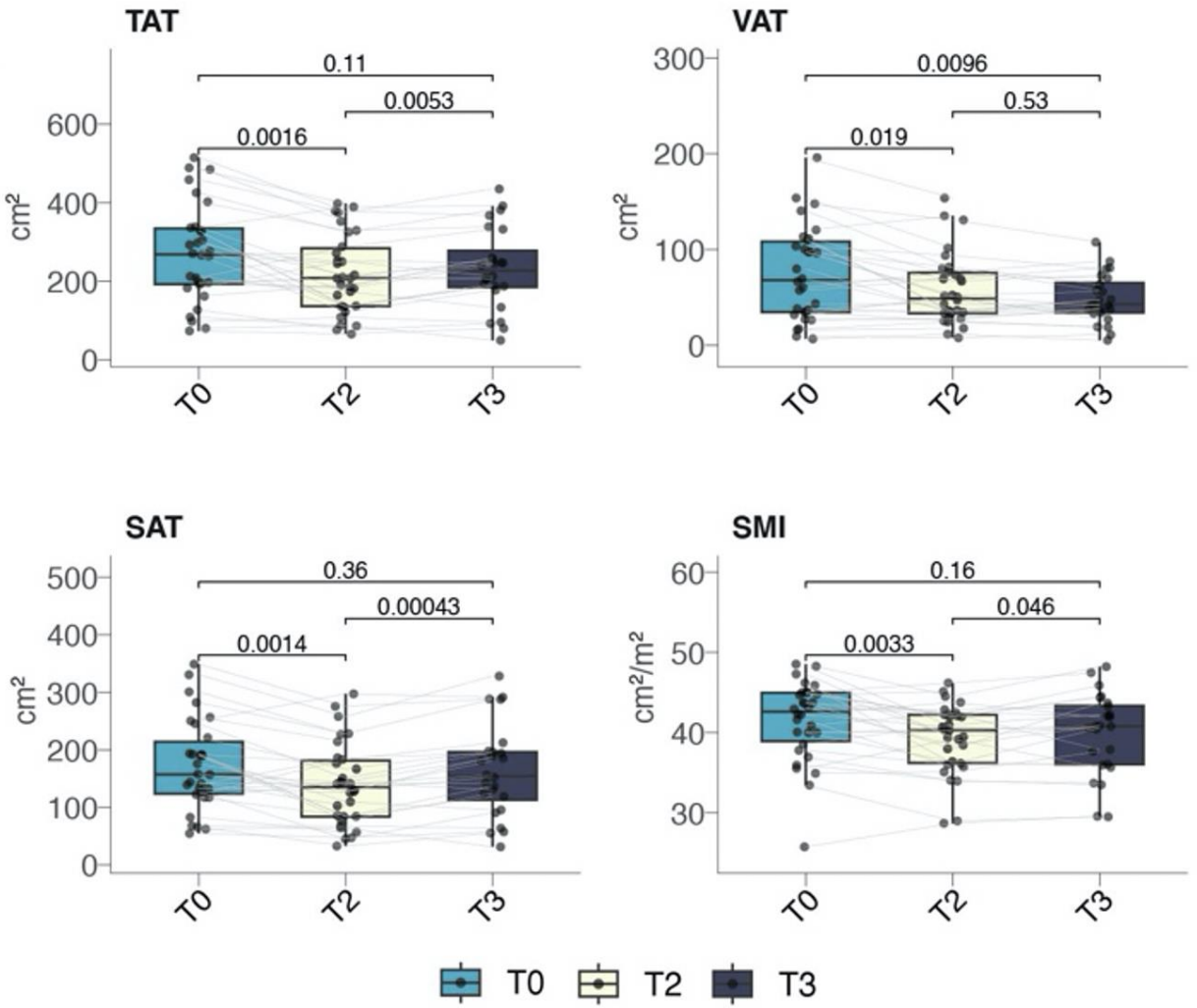


T0: baseline
T2: before surgery (end of therapy)

Ligorio F et al. Cell Metabolism 2025

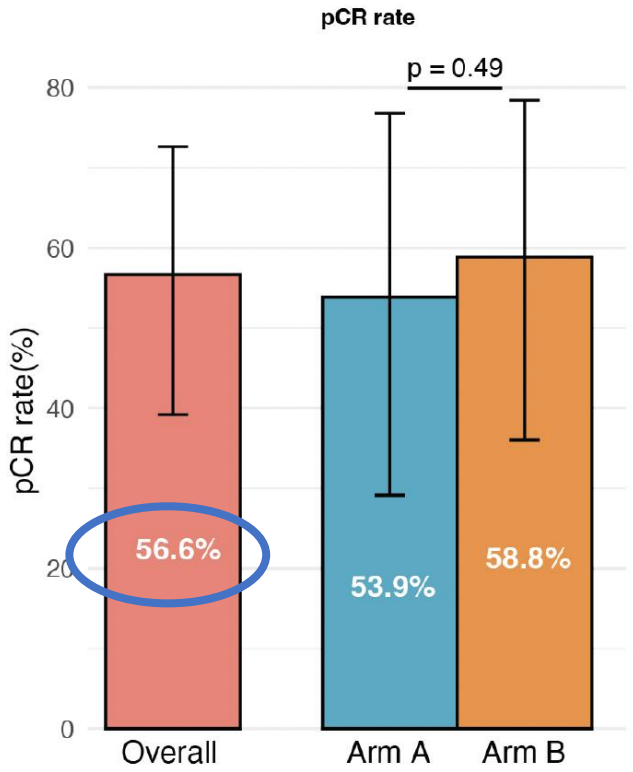
The FMD results in long-term reduction of visceral fat, while total fat tissue and skeletal muscle loss is partially restored





Ligorio F et al. Cell Metabolism 2025

Chemotherapy plus FMD is associated with excellent antitumor activity and efficacy regardless of metformin



Historical pCR rates[1,2,3]

30-39%

1. Alba E et al. Breast Cancer Res Treat 2012;136(2):487-93
2. Sikov WM et al. J Clin Oncol 2015;33(1):13-21
3. Loibl S et al. Lancet Oncol 2018;19(4):497-509

Vernieri C. SABCS 2023

Ligorio F et al. Cell Metabolism 2025

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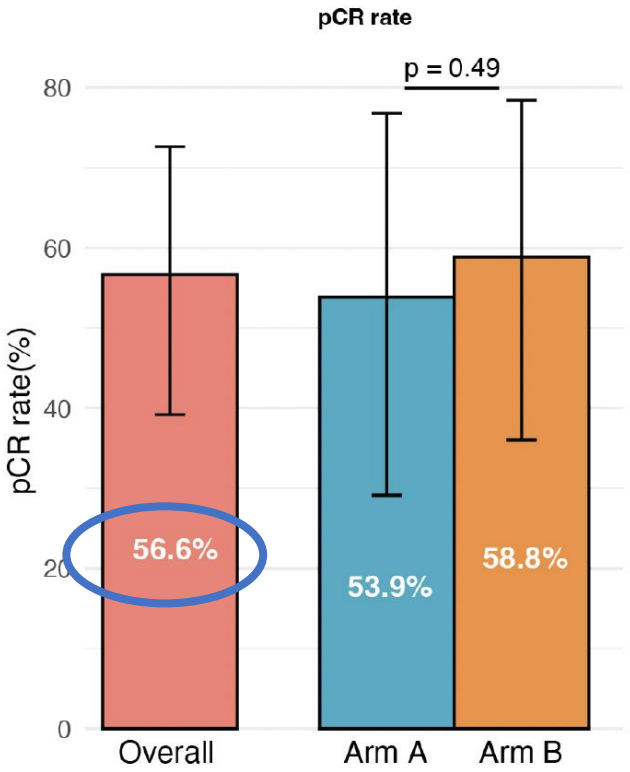

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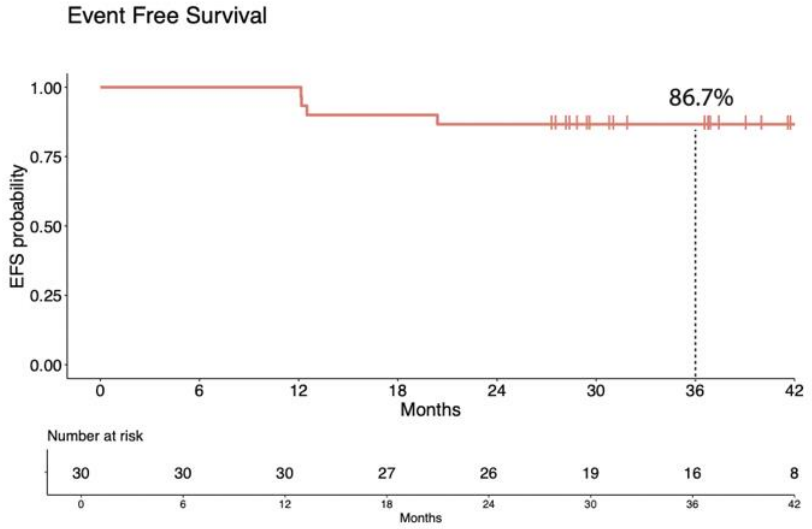

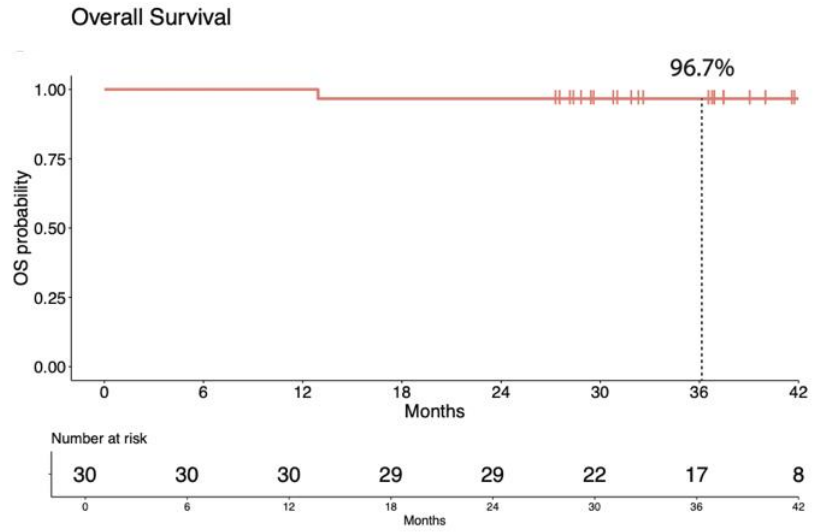

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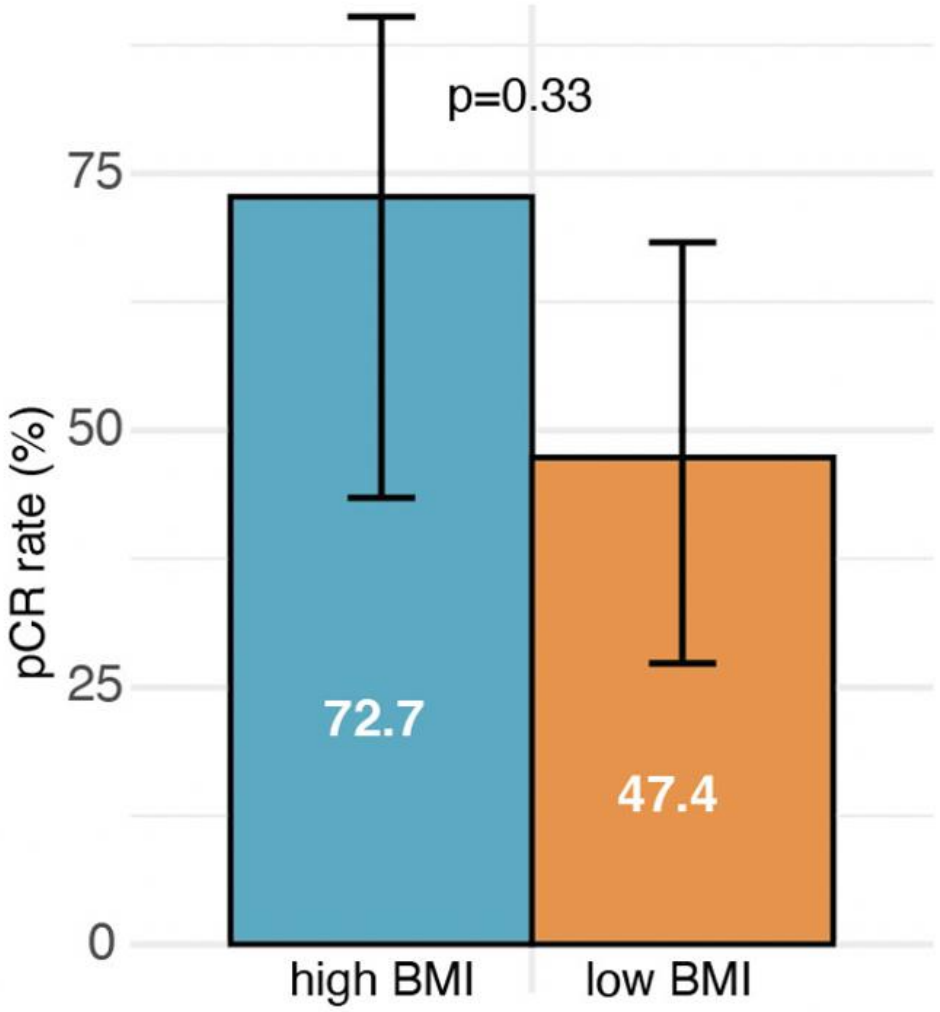
Vernieri C. SABCS 2023

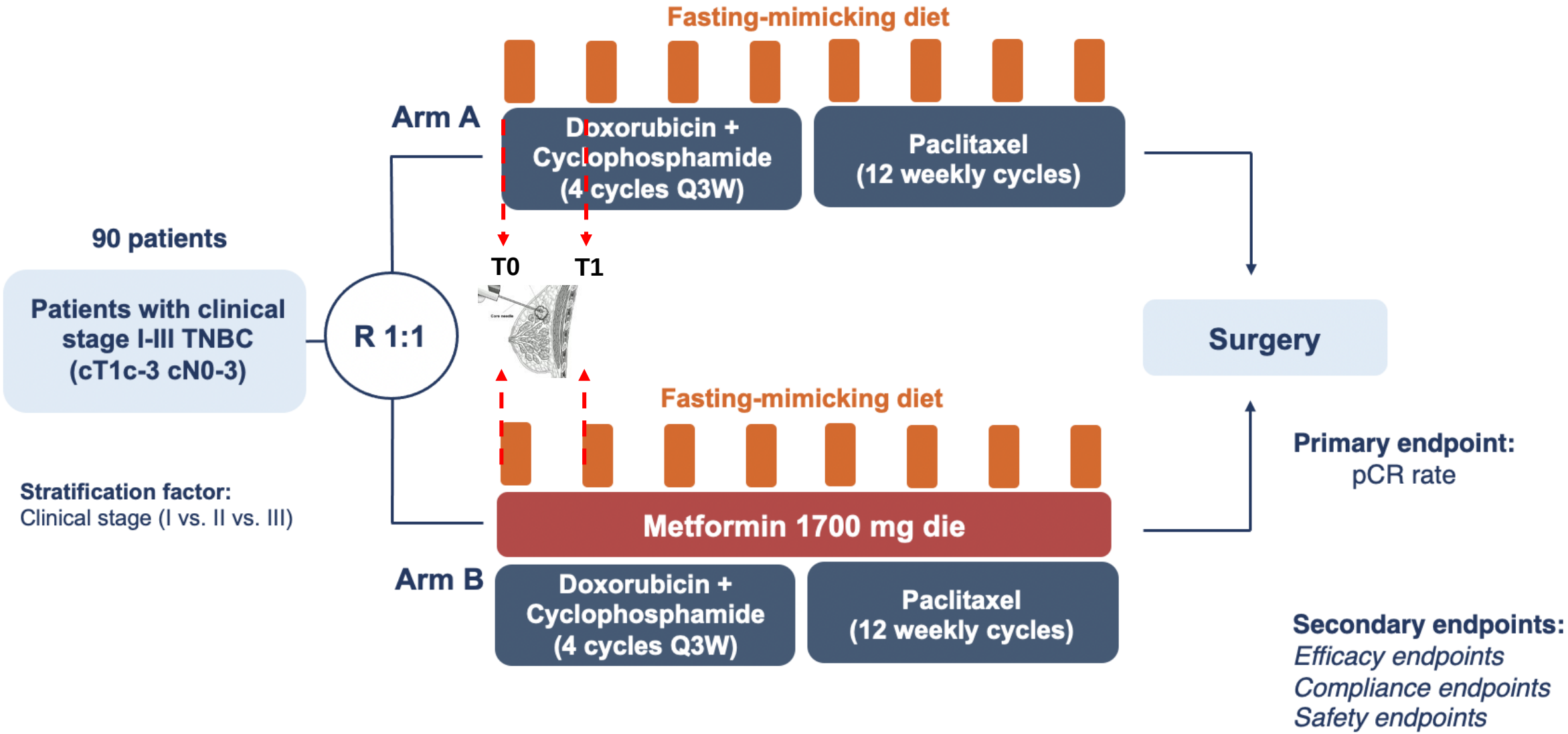
Ligorio F et al. Cell Metabolism 2025

The pCR rate was numerically higher in patients with higher baseline BMI



Ligorio F et al. Cell Metabolism 2025

Early modulation of TNBC metabolism during chemotherapy plus FMD



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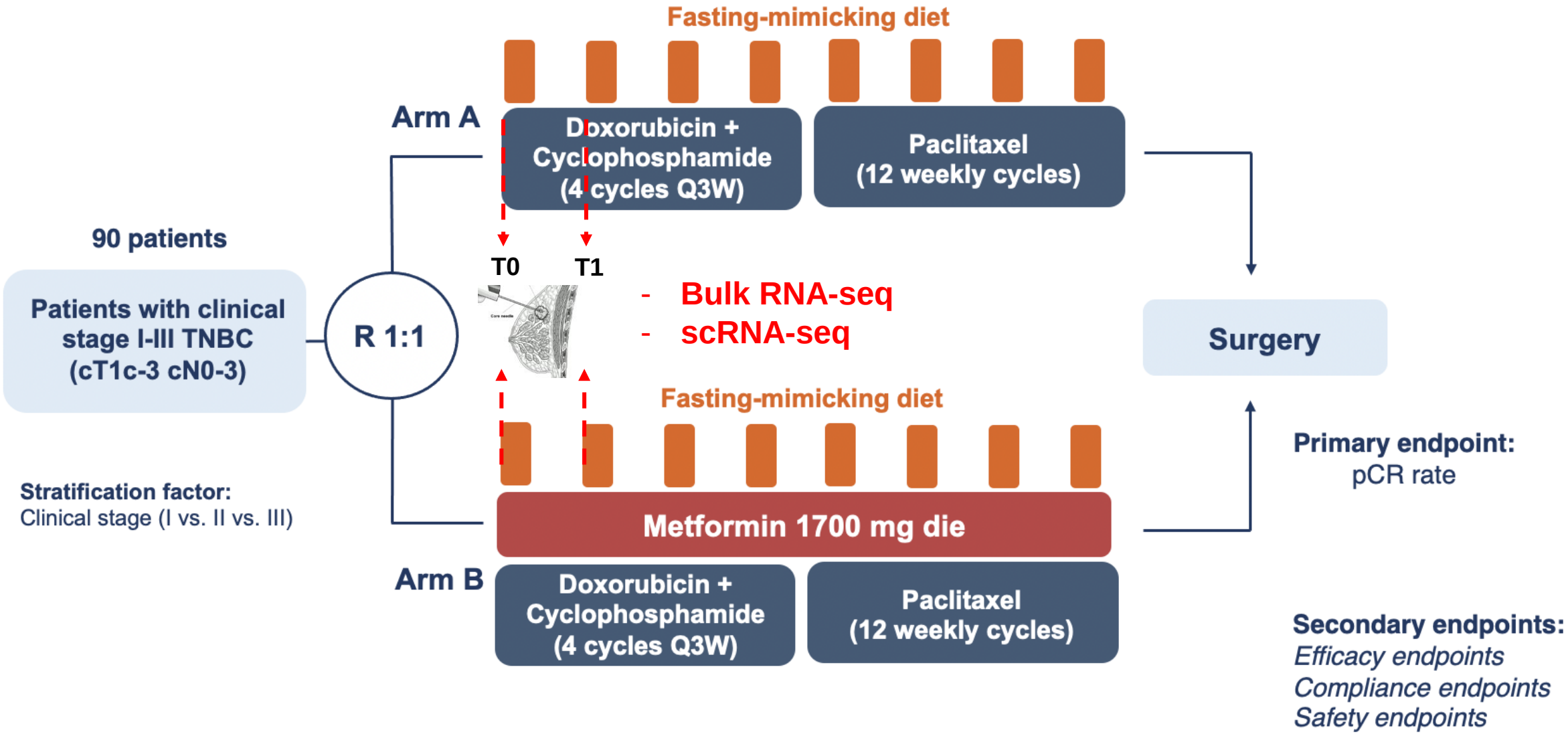
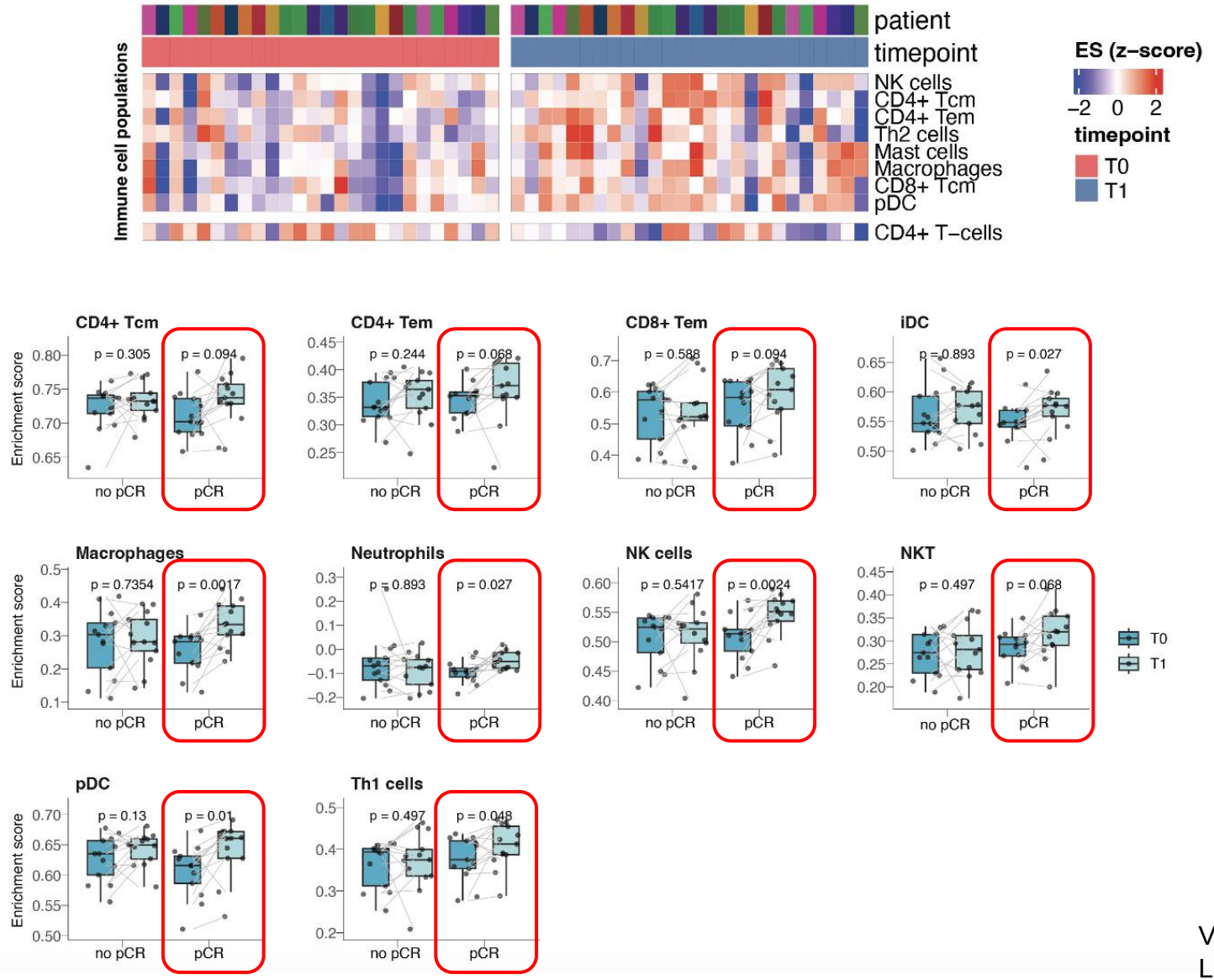


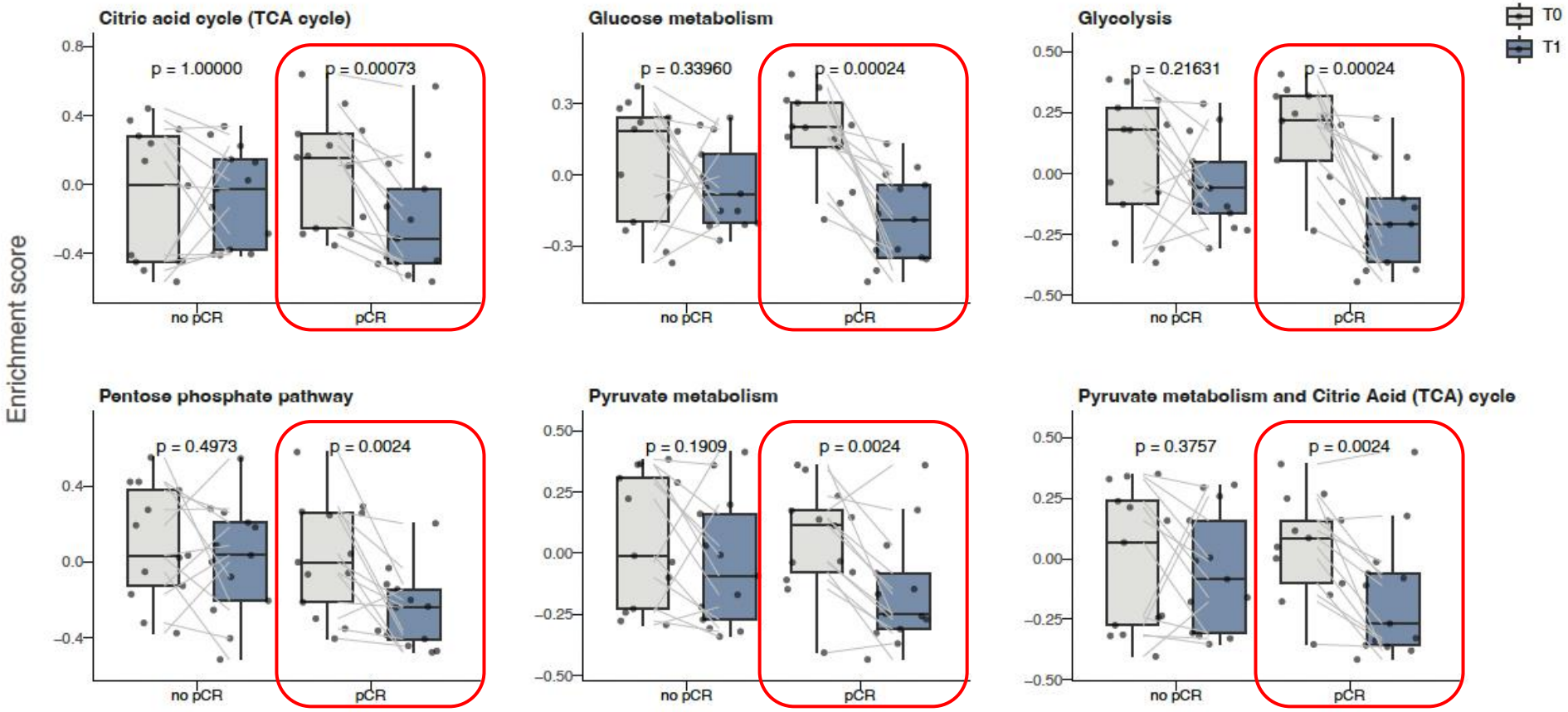
Several tumor-infiltrating immune cells are increased after the first treatment cycle only in patients achieving pCR



Vernieri C. SABCS 2023

Ligorio F et al. Cell Metabolism 2025

Glucose and pyruvate metabolism are only downregulated in tumors undergoing pCR

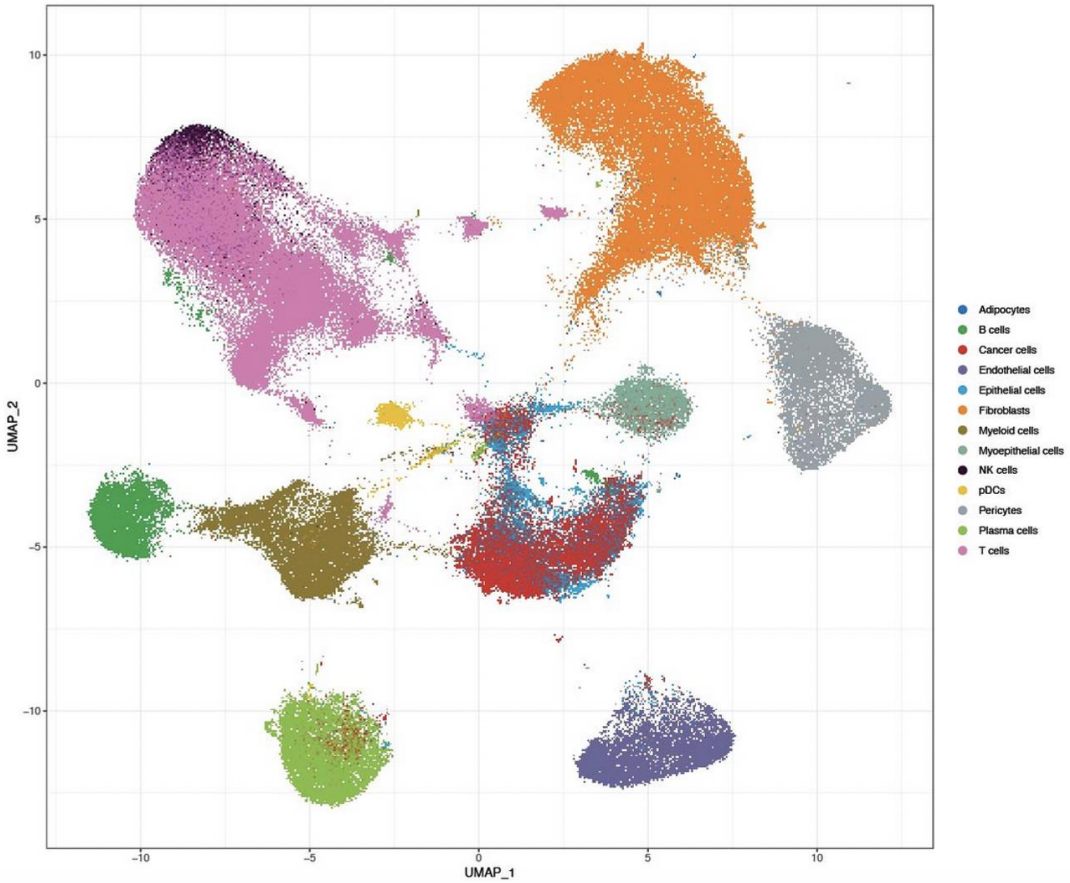


Vernieri C. SABCS 2023

Ligorio F et al. Cell Metabolism 2025

FMD downregulates glycolysis especially in more glycolytic cells in TNBC masses

UMAP of scRNAseq data and cell map



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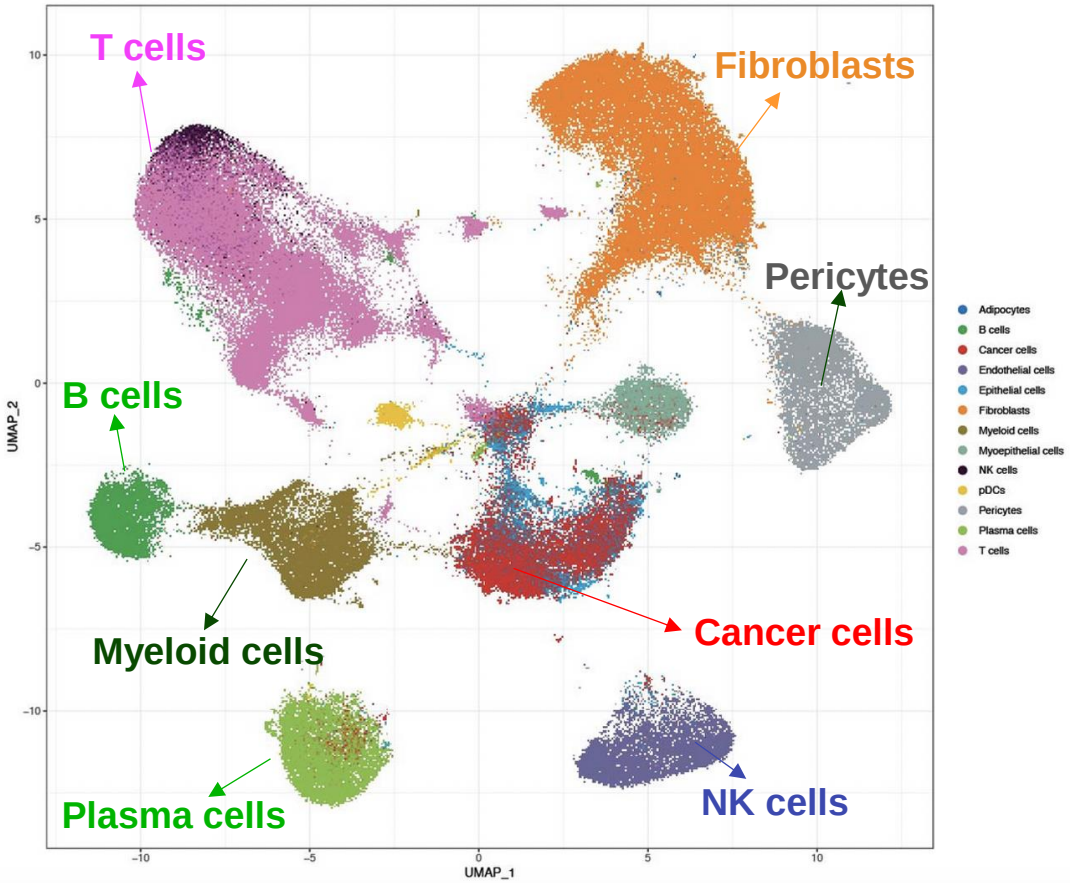

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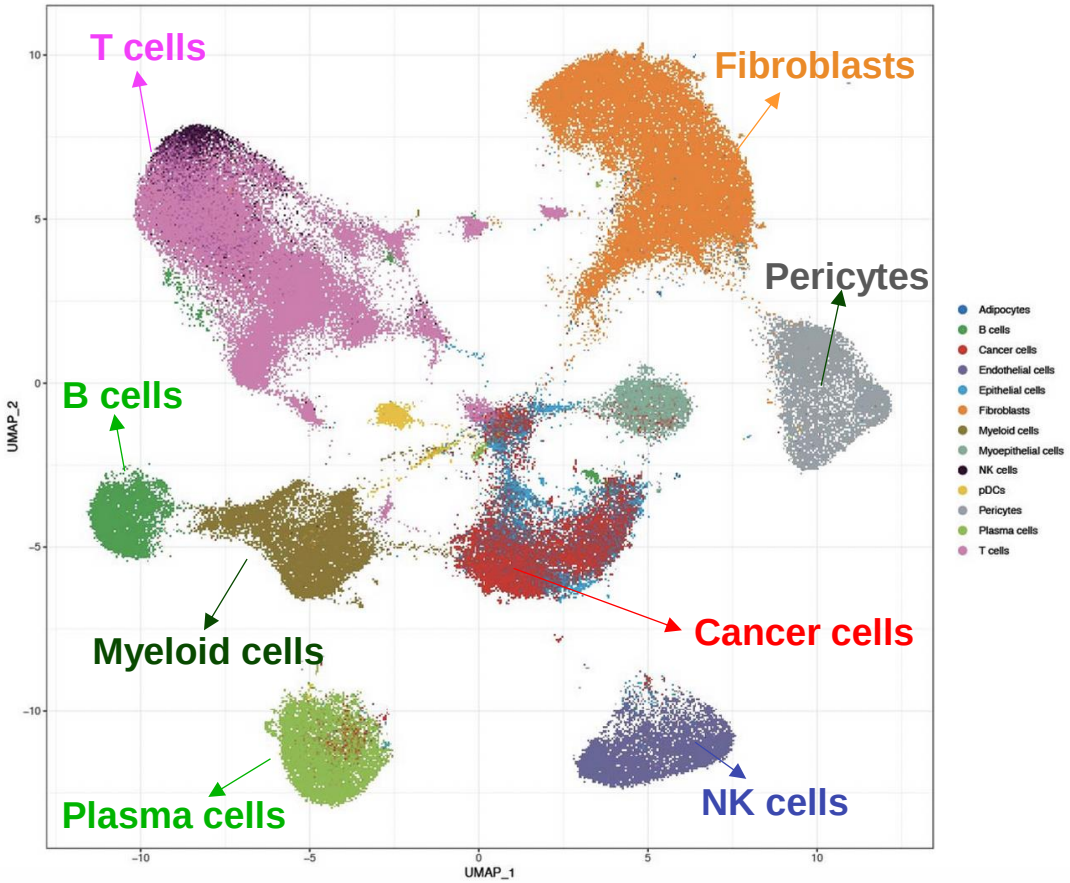

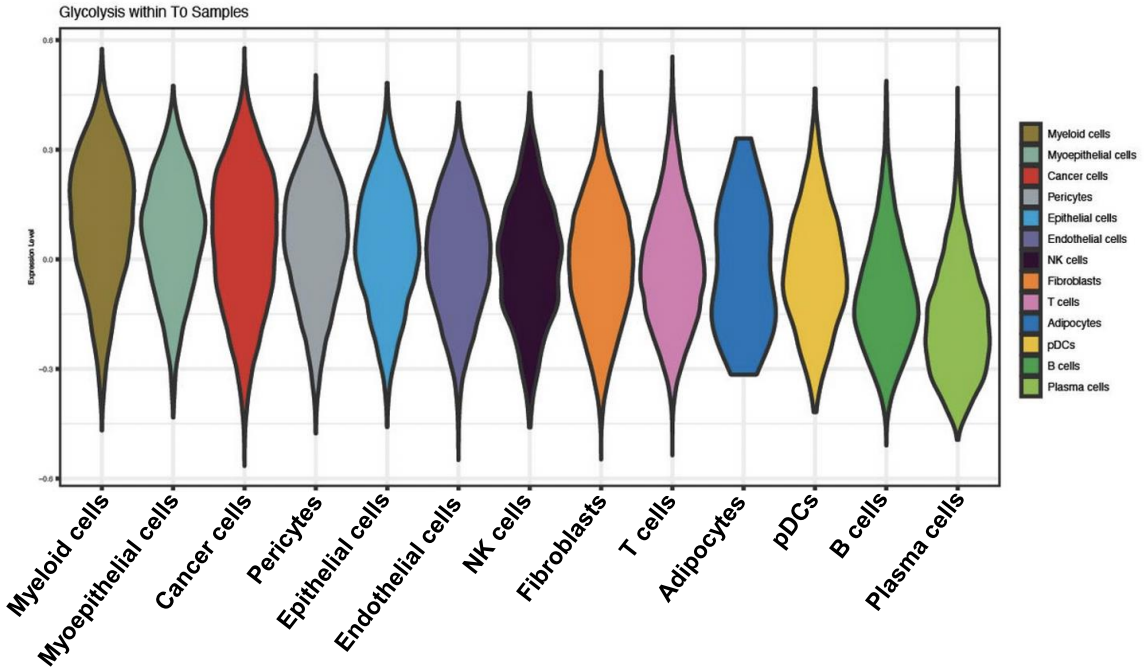

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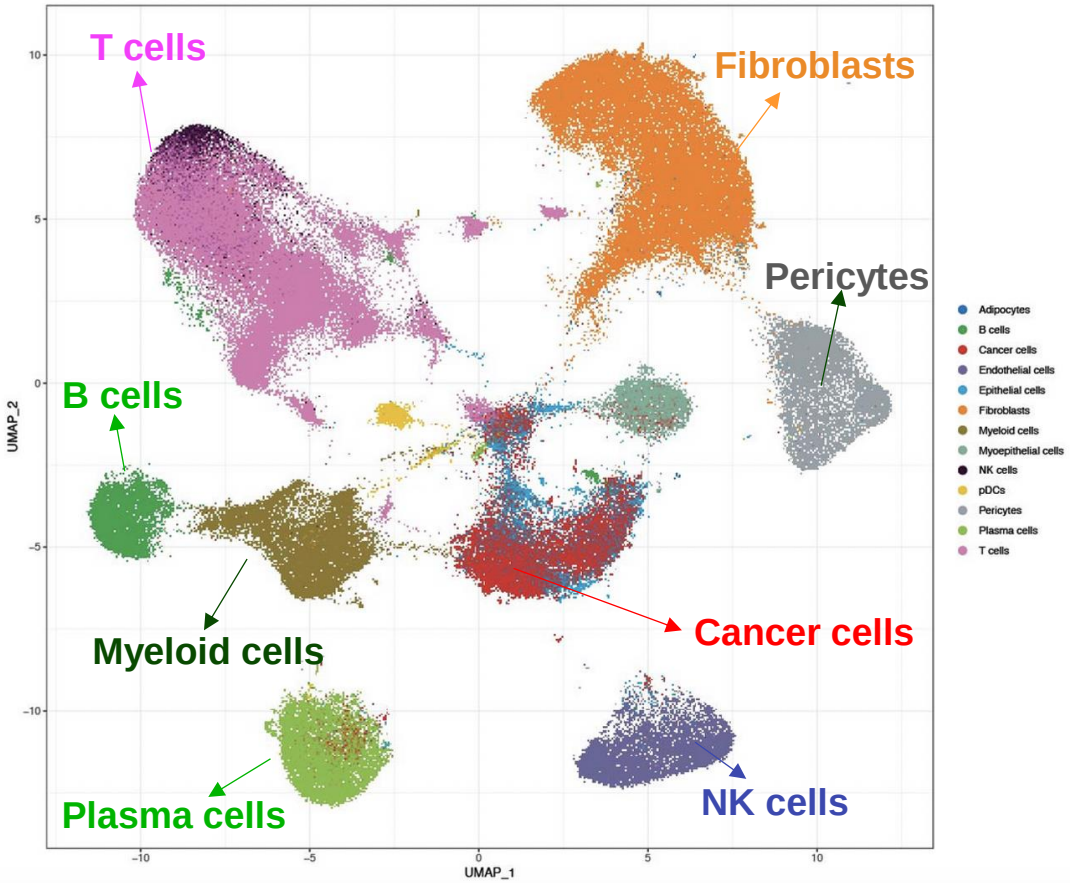
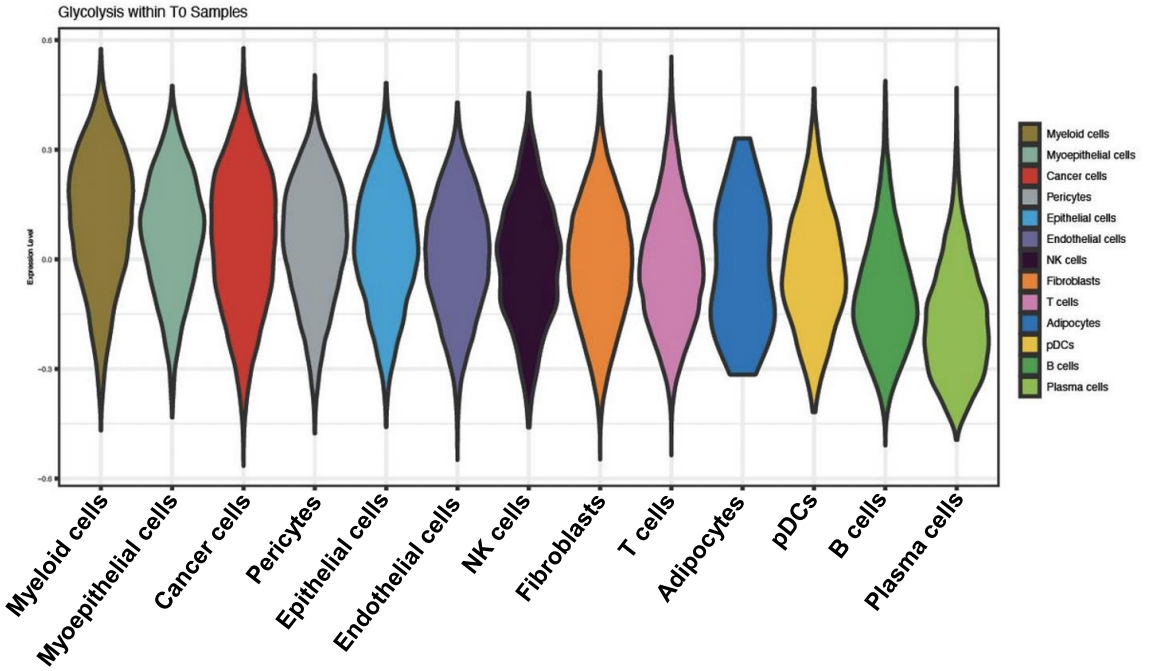
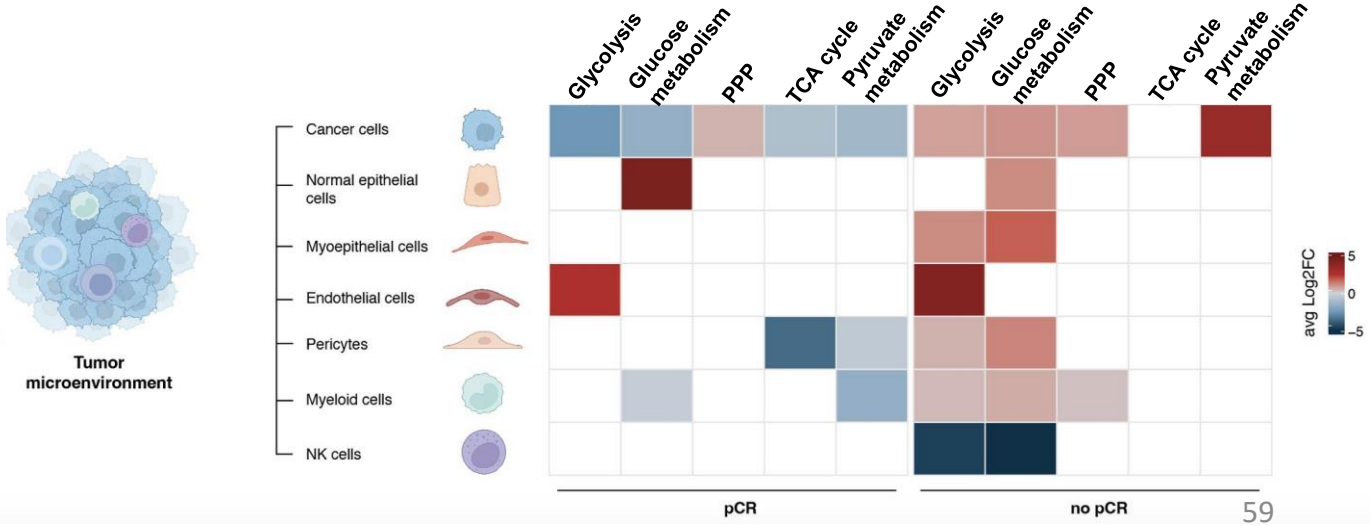
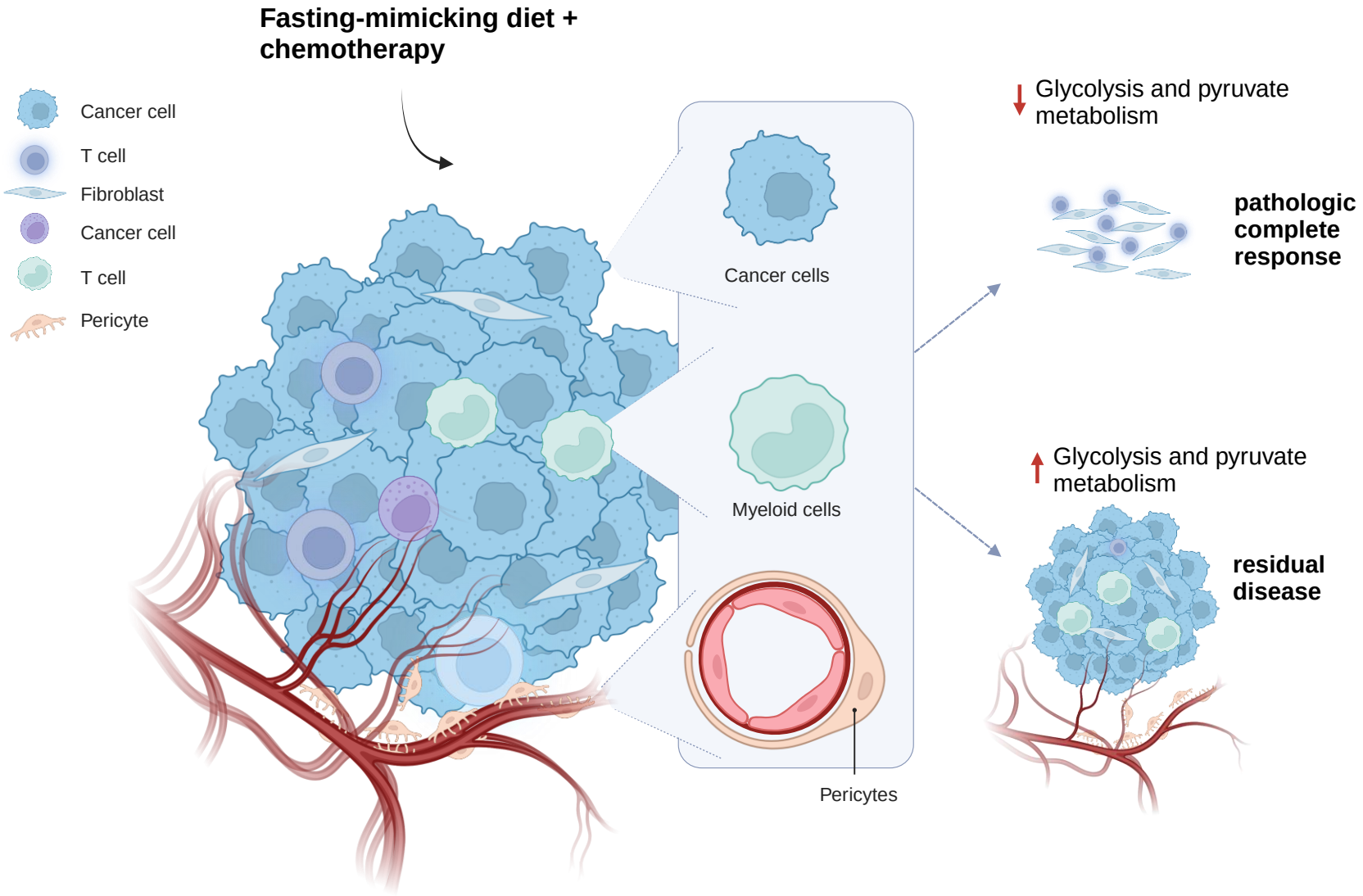






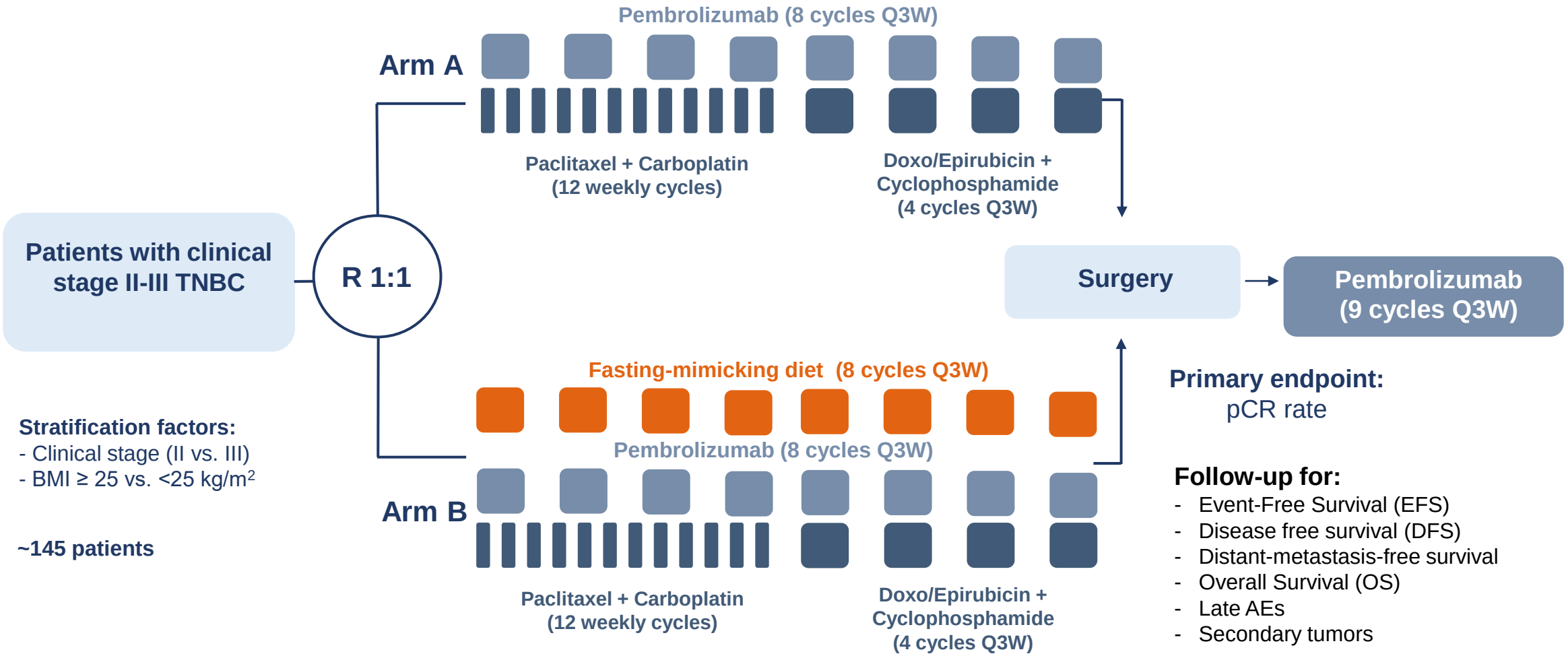
Ligorio F et al. Cell Metabolism 2025

Model of FMD plus chemotherapy effects in human TNBCs



Ligorio F et al. Cell Metabolism 2025

BREAKFAST 2 trial (NCT05763992)



Stratification factors:
- Clinical stage (II vs. III)
- BMI ≥ 25 vs. <25 kg/m^2

~145 patients

Primary endpoint:
pCR rate

Follow-up for:
- Event-Free Survival (EFS)
- Disease free survival (DFS)
- Distant-metastasis-free survival
- Overall Survival (OS)
- Late AEs
- Secondary tumors

BREAKFAST 2 trial: participating centers

N=24 patients enrolled (22 at INT)

BREAKFAST 2 trial: translational platform

Patients with clinical stage II-III TNBC

R 1:1

Arm A

Pembrolizumab (8 cycles Q3W)

Paclitaxel + Carboplatin (12 weekly cycles)

Doxo/Epirubicin + Cyclophosphamide (4 cycles Q3W)

Arm B

Fasting-mimicking diet (8 cycles Q3W)

Pembrolizumab (8 cycles Q3W)

Paclitaxel + Carboplatin (12 weekly cycles)

Doxo/Epirubicin + Cyclophosphamide (4 cycles Q3W)

Surgery

Pembrolizumab (9 cycles Q3W)

Primary endpoint:
pCR rate

Stratification factors:
- Clinical stage (II vs. III)
- BMI ≥ 25 vs. <25 kg/m^2

~145 patients

Tumor Samples

Baseline

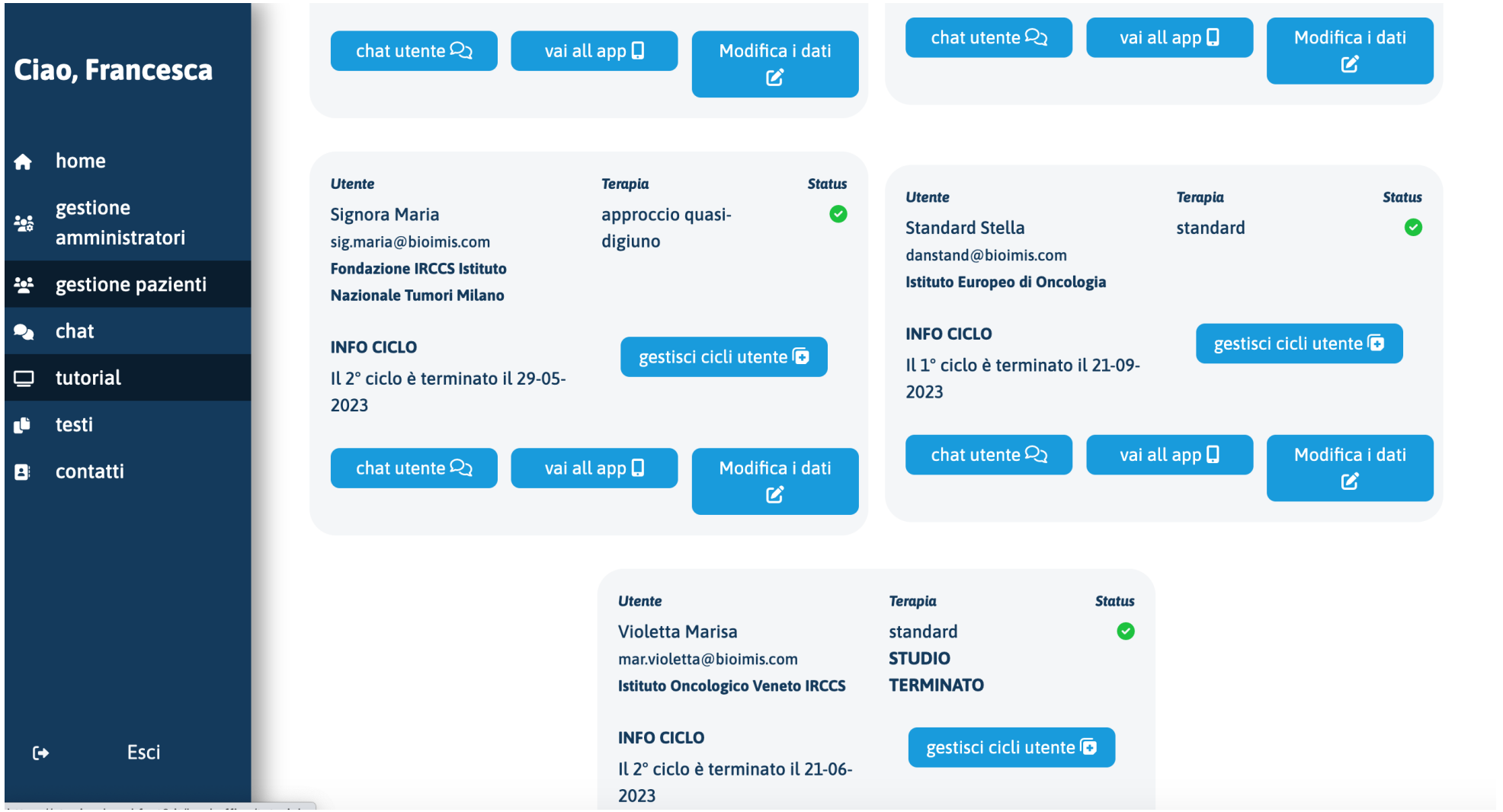
Post-C1 (optional)

Surgery

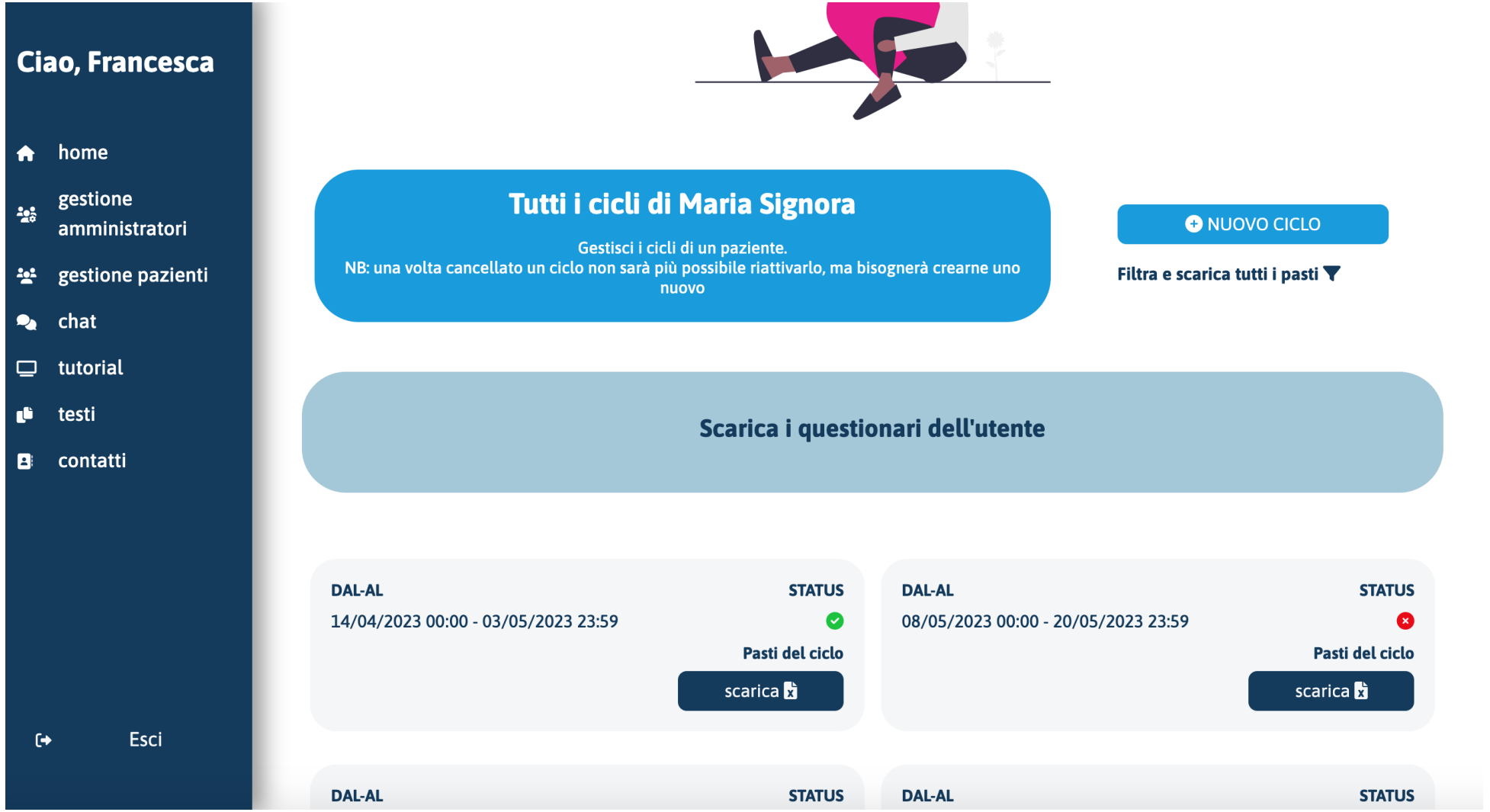
Blood/fecal/saliva samples

C1 **C2** **C5** **Surgery** **Adjuvant phase**

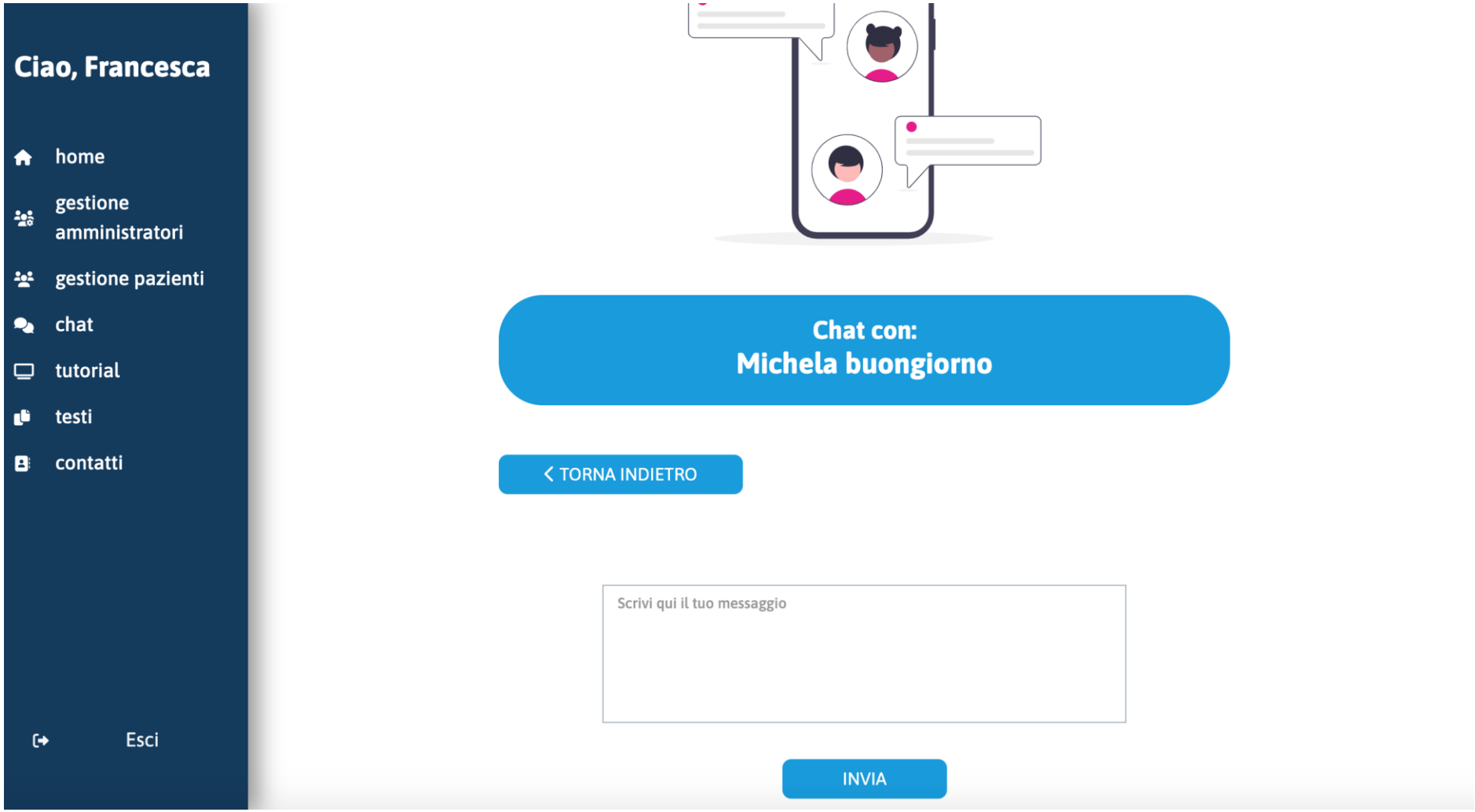
A WebApp to follow patients from other centers



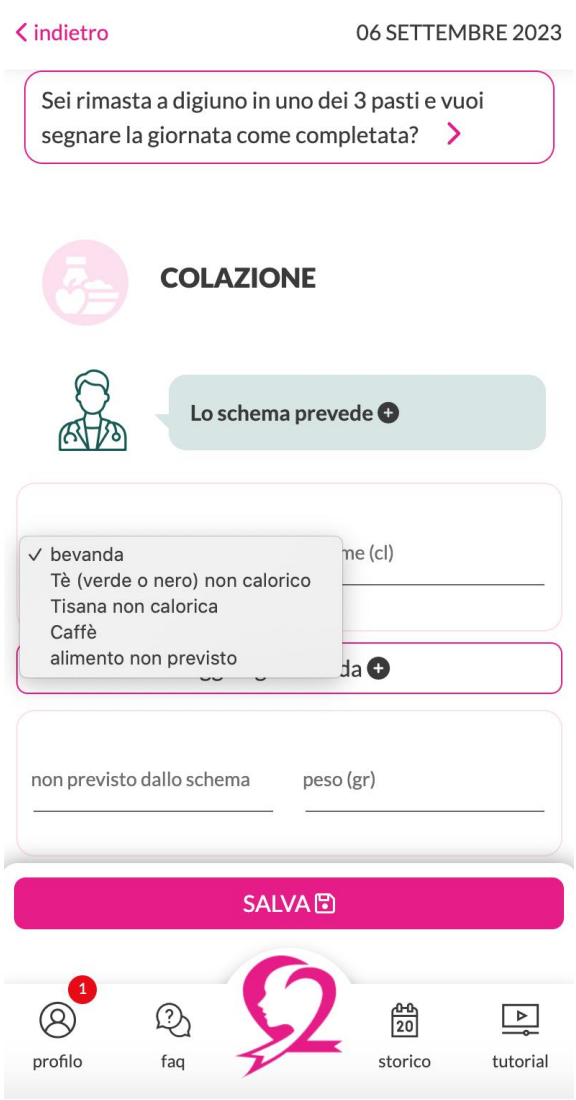
WebApp interface

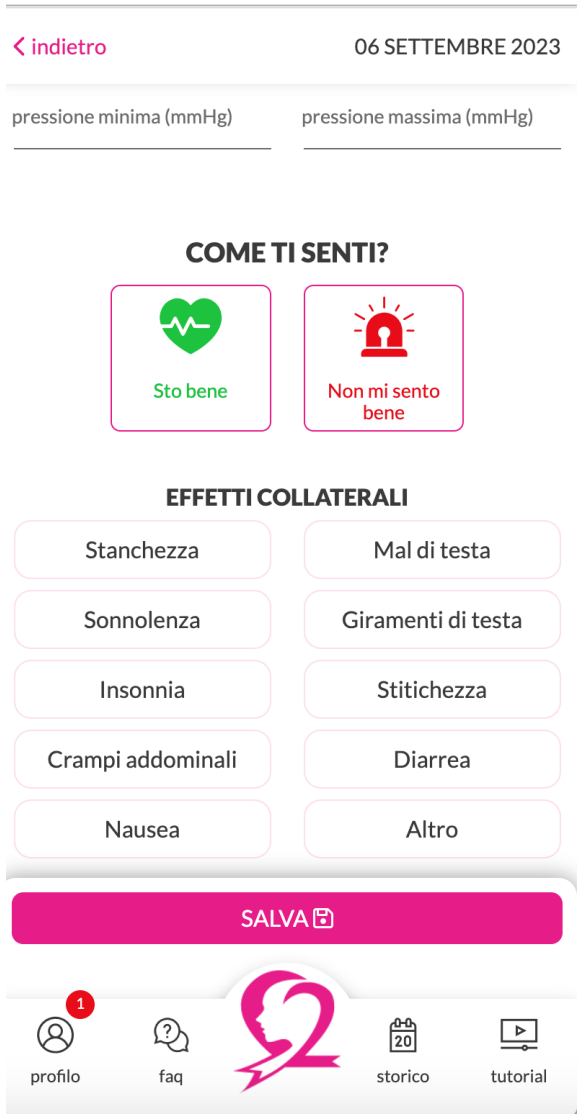


WebApp: an open chat with patients

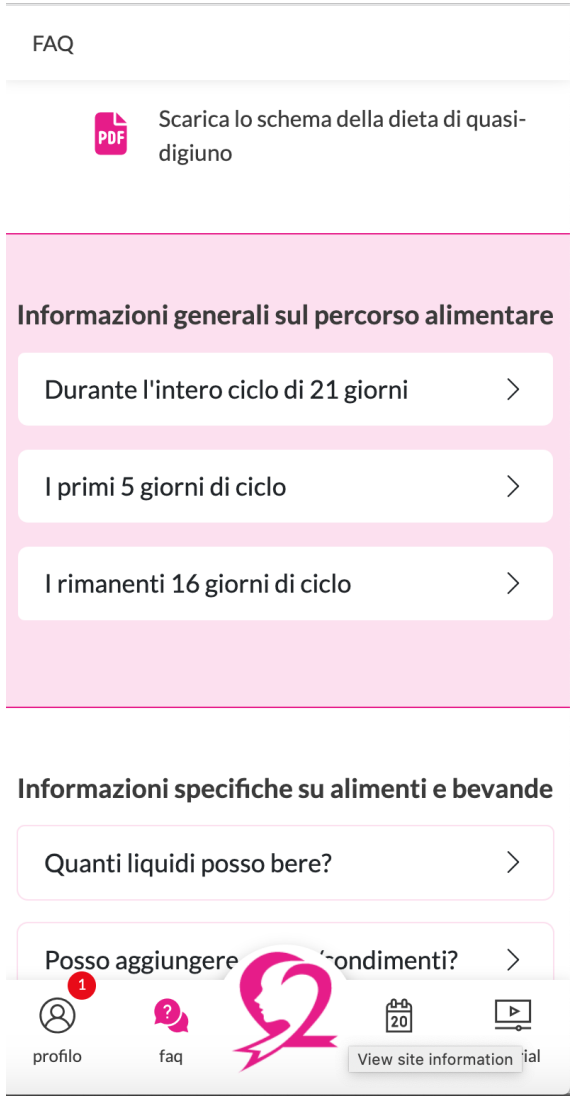


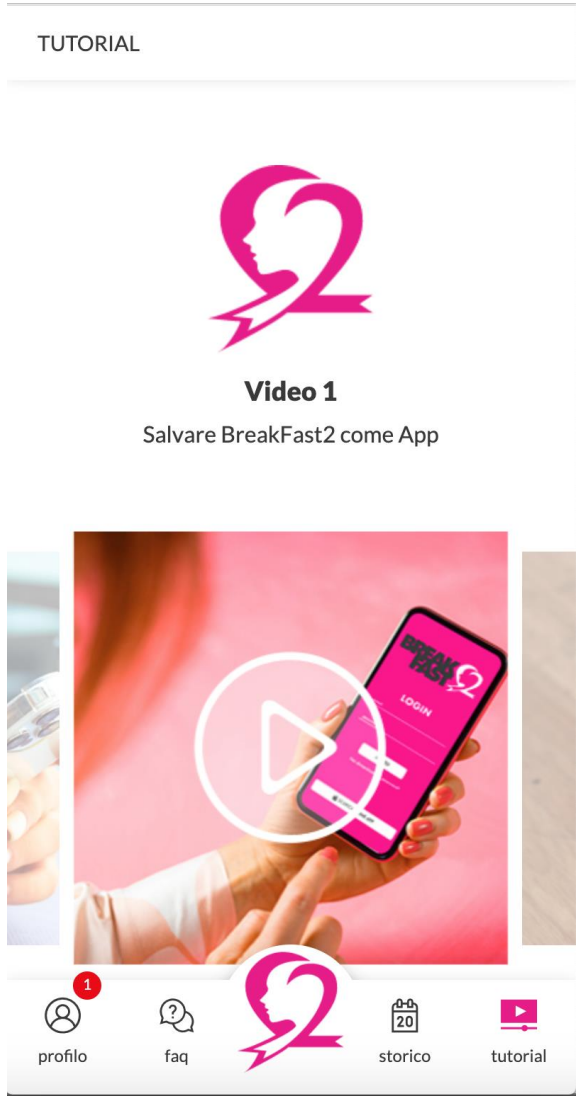
Daily management of patients: how the chat helps



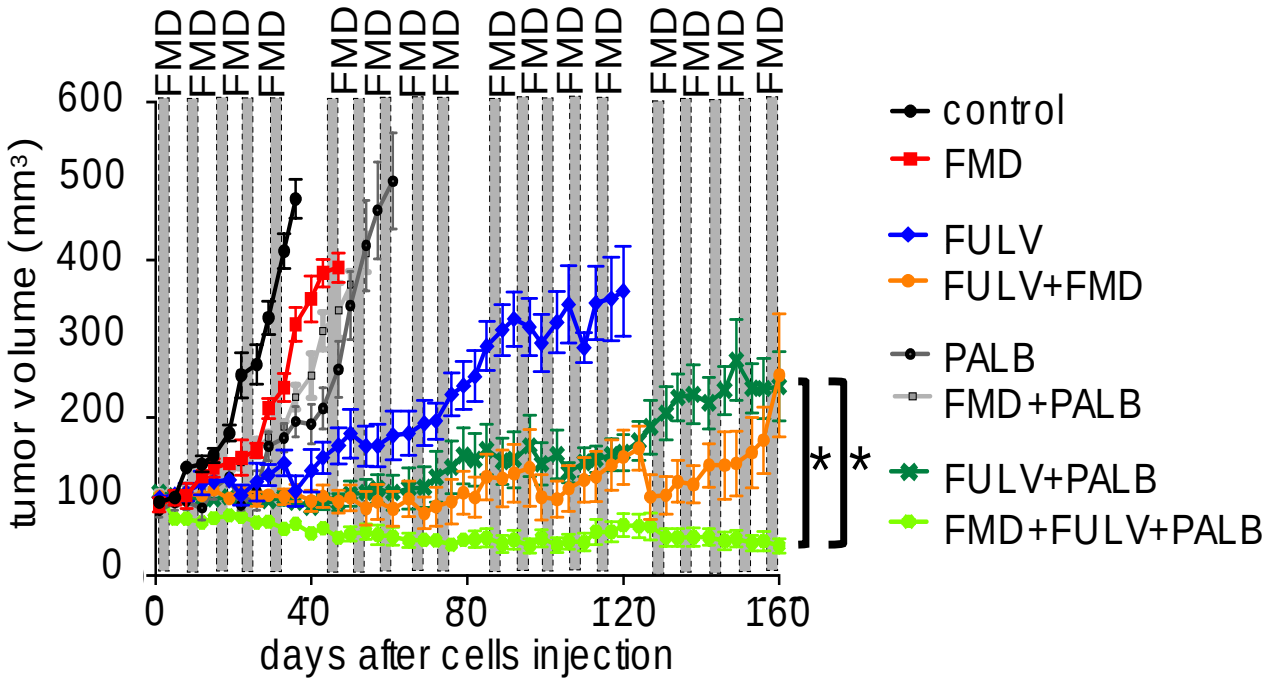


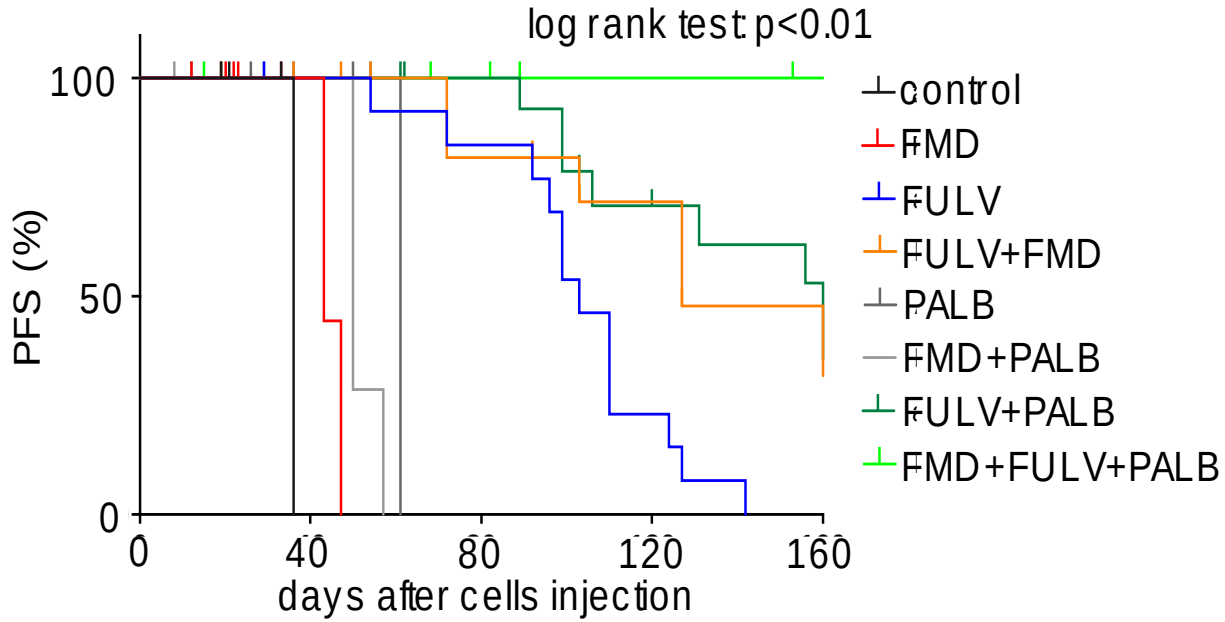
Daily management of patients: how the WebApp helps





FMD in combination with Fulvestrant plus Palbociclib results in long term inhibition of *in vivo* tumor growth and in prolongation of animal survival

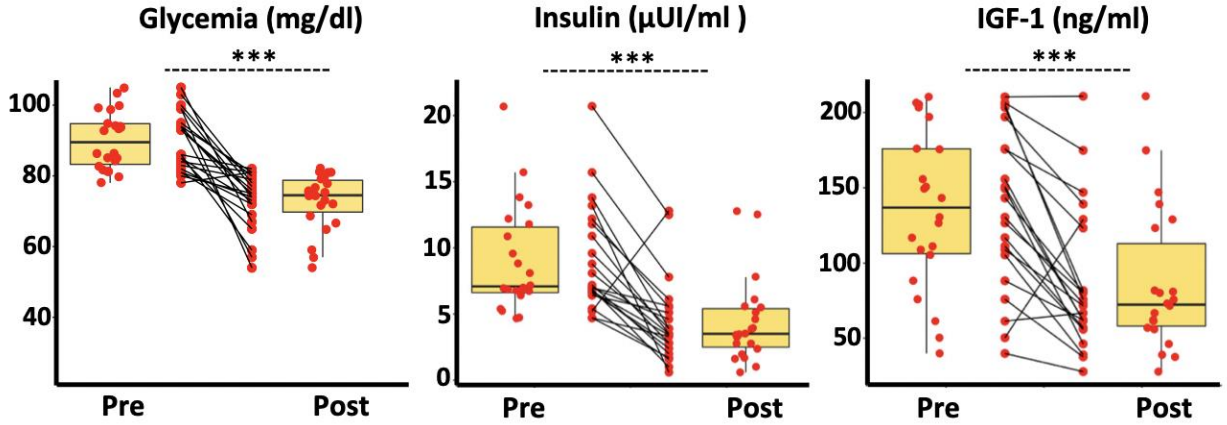


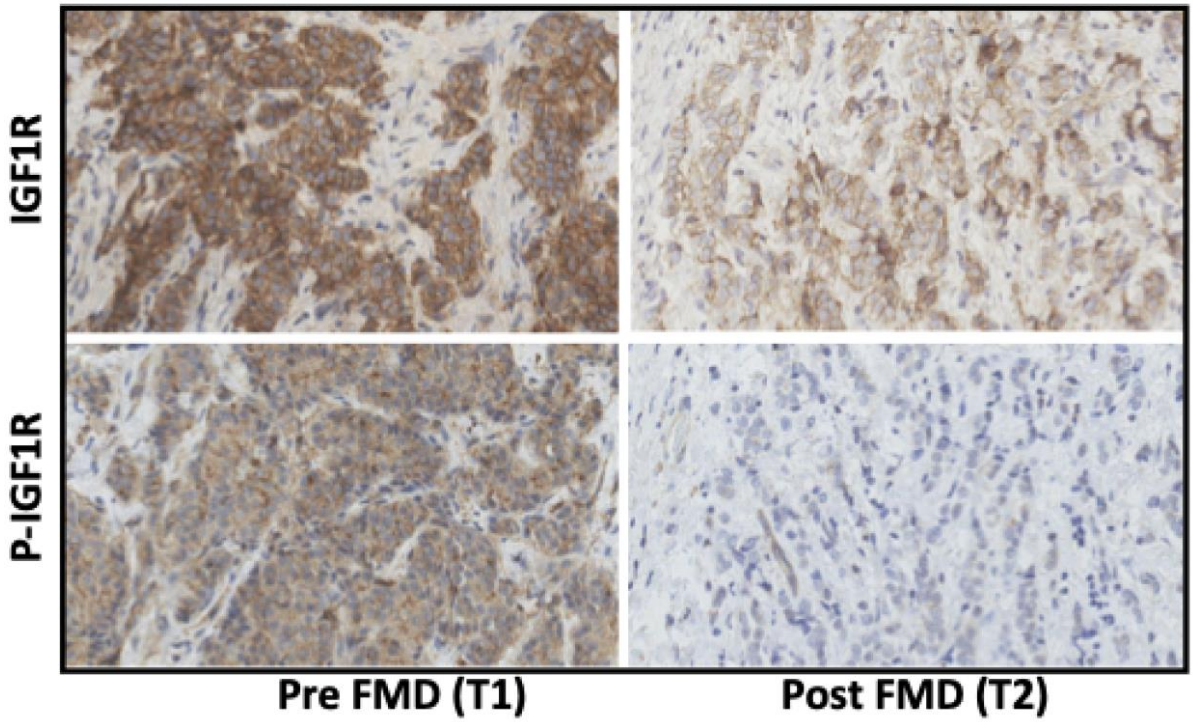


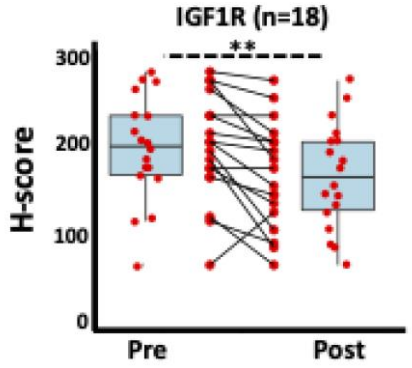
MCF-7 mouse xenografts

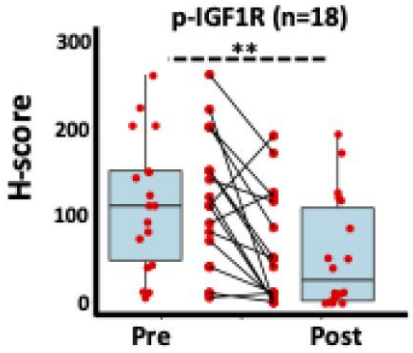
Caffa I, Spagnolo V et al. Nature 2020

Severe calorie restriction reduces blood IGF-1 levels and intratumor expression of IGF1R and phosphorylated (activated) IGF1R in breast cancer patients









Vernieri C et al. Cancer Discov 2022

FASTENCICLIB: a phase II, multicenter, single arm trial to investigate the efficacy of calorie restriction with first-line ET+CDK4/6i in patients with HR+/HER2- aBC

Study design

aHR+ HER2-

Main inclusion criteria:
- No previous treatment for advanced disease
- ECOG PS 0-2
- BMI > 19 Kg/m^2

Experimental treatment

ET* + CDK4/6i

+ 5-day calorie restriction cycles (q28) #

Until PD or unacceptable toxicity

Primary endpoint: clinical (12 month PFS)

Primary endpoint: PFS rate at 12 months

Secondary endpoints: OS, PFS, ORR, safety of the experimental regimen

*NSAI or fulvestrant #Each calorie restriction cycle consists of 5 consecutive days (on days 1 through 5 of each cycle) of a plant-based, low-calorie (about 600 Kcal on day 1; about 300 Kcal on day 2 to 5), low-protein, low-carbohydrate diet.

Enrollment initiation: June 2024

N=152 patients (multicentric)

Different nutritional interventions

- Nutrient deprivation

- **Nutrient supplementation**

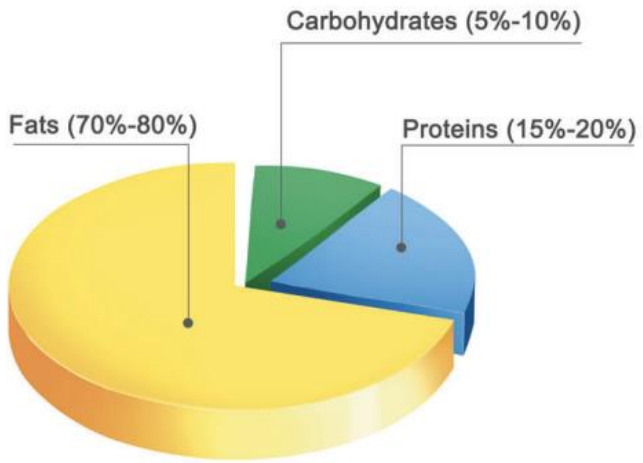
Nutrient supplementation approaches

- Ketogenic diets
- Amino acid supplementation
- Unsaturated fatty acid supplementation

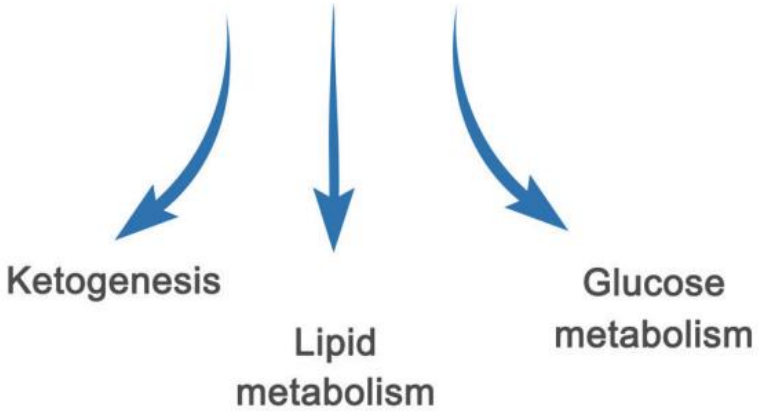
Nutrient supplementation approaches

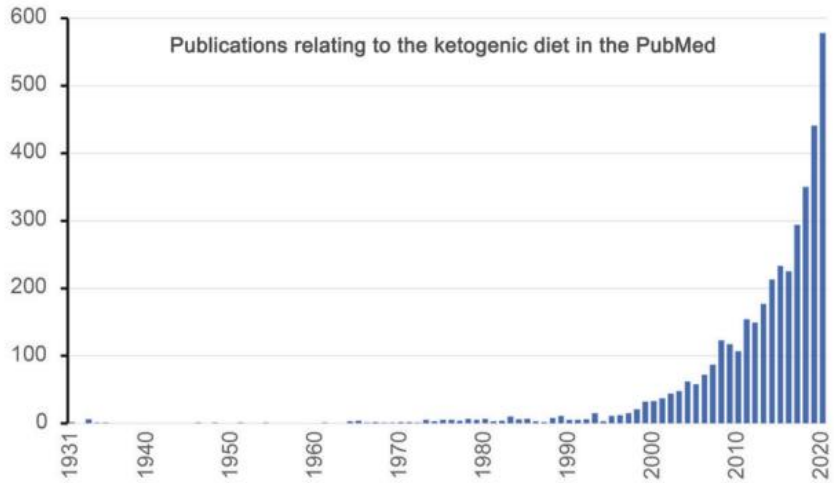
- **Ketogenic diets**
- Amino acid supplementation
- Unsaturated fatty acid supplementation

Ketogenic diet: macronutrient composition



Classic KD (Long-chain triglycerides,LCT)	4:1 (3:1-4:1)	Fat (grams) to protein + carbohydrate (grams)
MCT KD (Medium- chain triglyceride)	30%-60%	Energy from MCT
MAD (Modified Atkins diet)	1:1-1.5:1	Ketogenetic ratio
LGIT (Low glycemic index treatment)	<50	Glycemic indices for carbohydrates

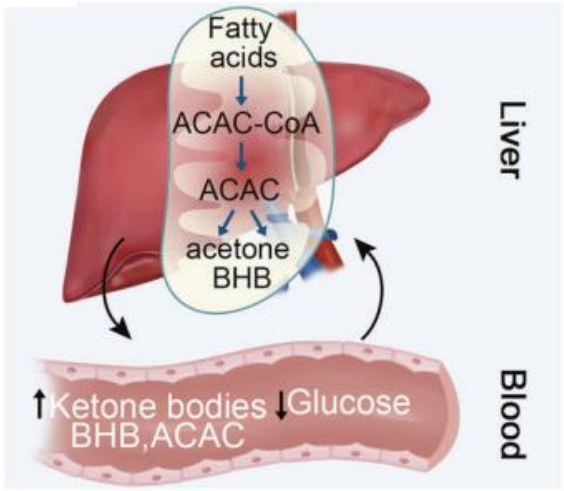


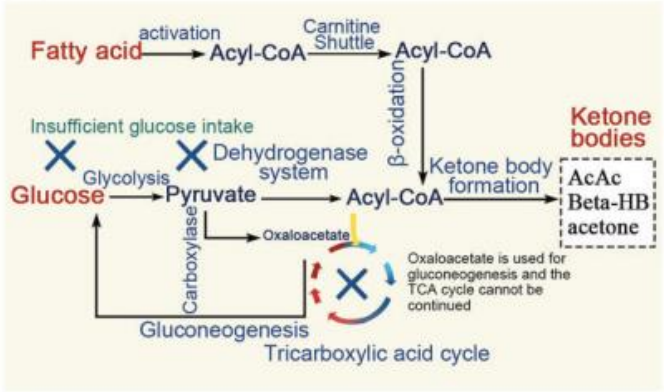


Zhu H et al. Signal Tranduct Target Ther 2022

Impact of ketogenic diets on systemic metabolism

Ketogenic diet and metabolism



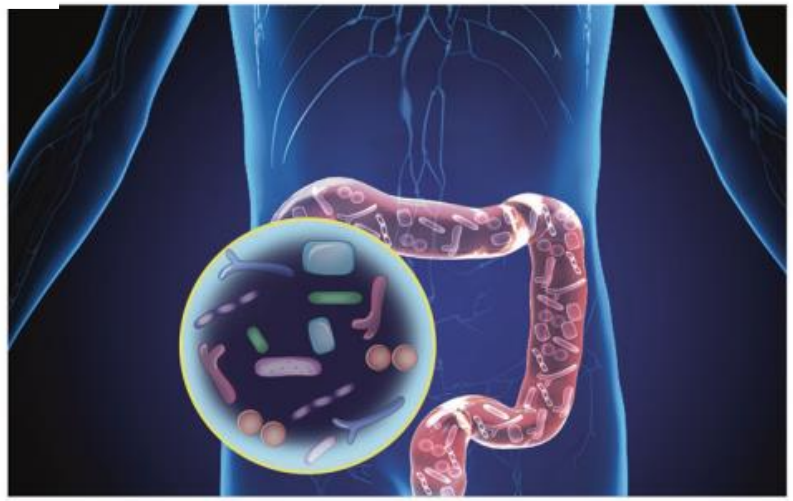


FA,and Ketone bodies ↑ **Glucose ↓**

- Cholesterol ↓
- TGs ↓
- HDL ↑
- Size and volume of LDL ↑
- Fibroblast growth factor 1 ↓
- HMG-CoA reductase ↑
- PPARα ↑
- Whole-body fatty acid oxidation ↑
- Liver ketogenesis ↑

- β-HB,AcAc ↑
- Insulin sensitivity ↑
- Mitochondrial oxidation ATP ↑
- The ratio of insulin to glucagon ↓
- Risk of type 2 diabetes ↓

Ketogenic diet and gut microbiota



Composition and diversity

- Bifidobacteria ↓
- Desulfovibrio ↓
- Turicibacter ↓
- Escherichia ↓
- Salmonella ↓
- Vibrio ↓
- Overall diversity ↓
- Akkermansia muciniphila ↑
- Parabacteriodes ↑
- Lactobacillus ↑
- Ruminococcaceae ↑
- Bacteroidetes ↑
- Roseburia ↑

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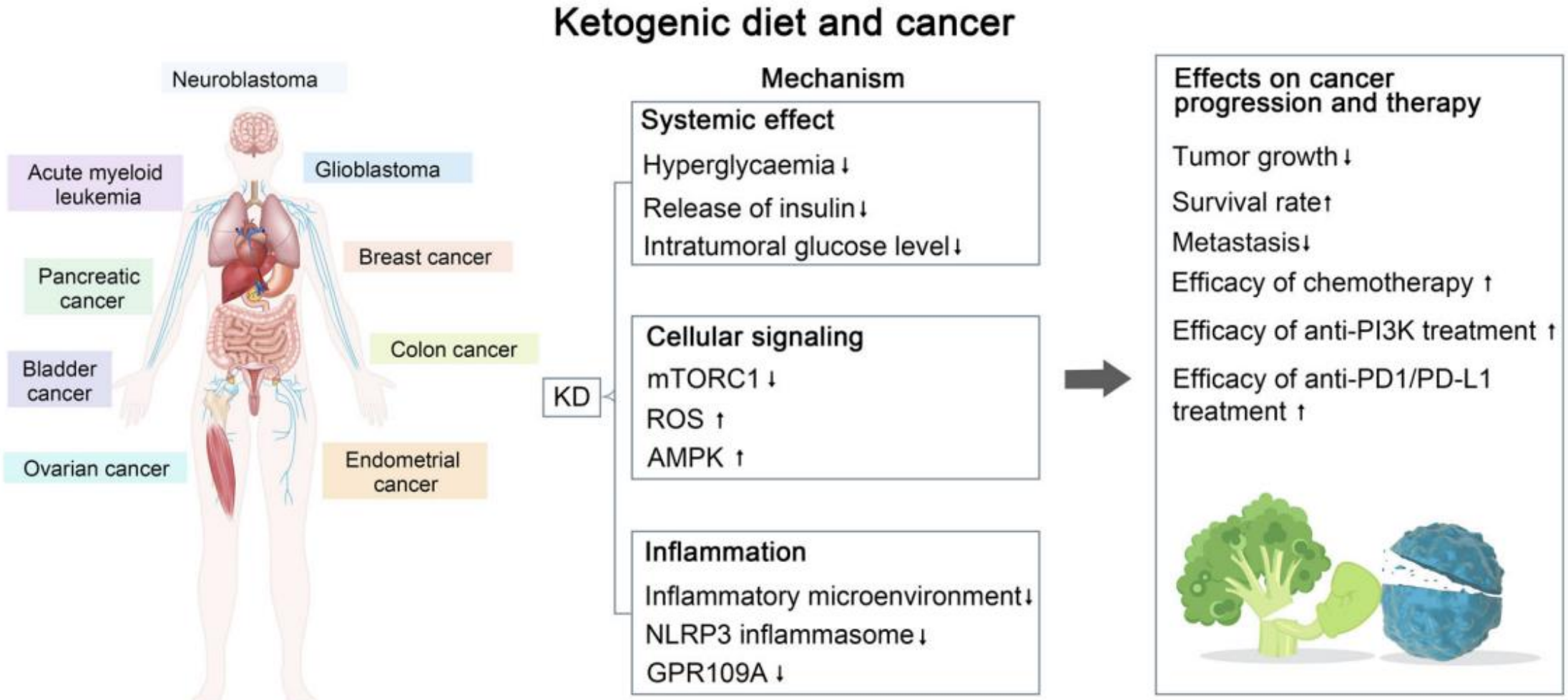
Ketogenic diets and cancer: results of preclinical studies



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Clinical evidence regarding ketogenic diets in cancer patients

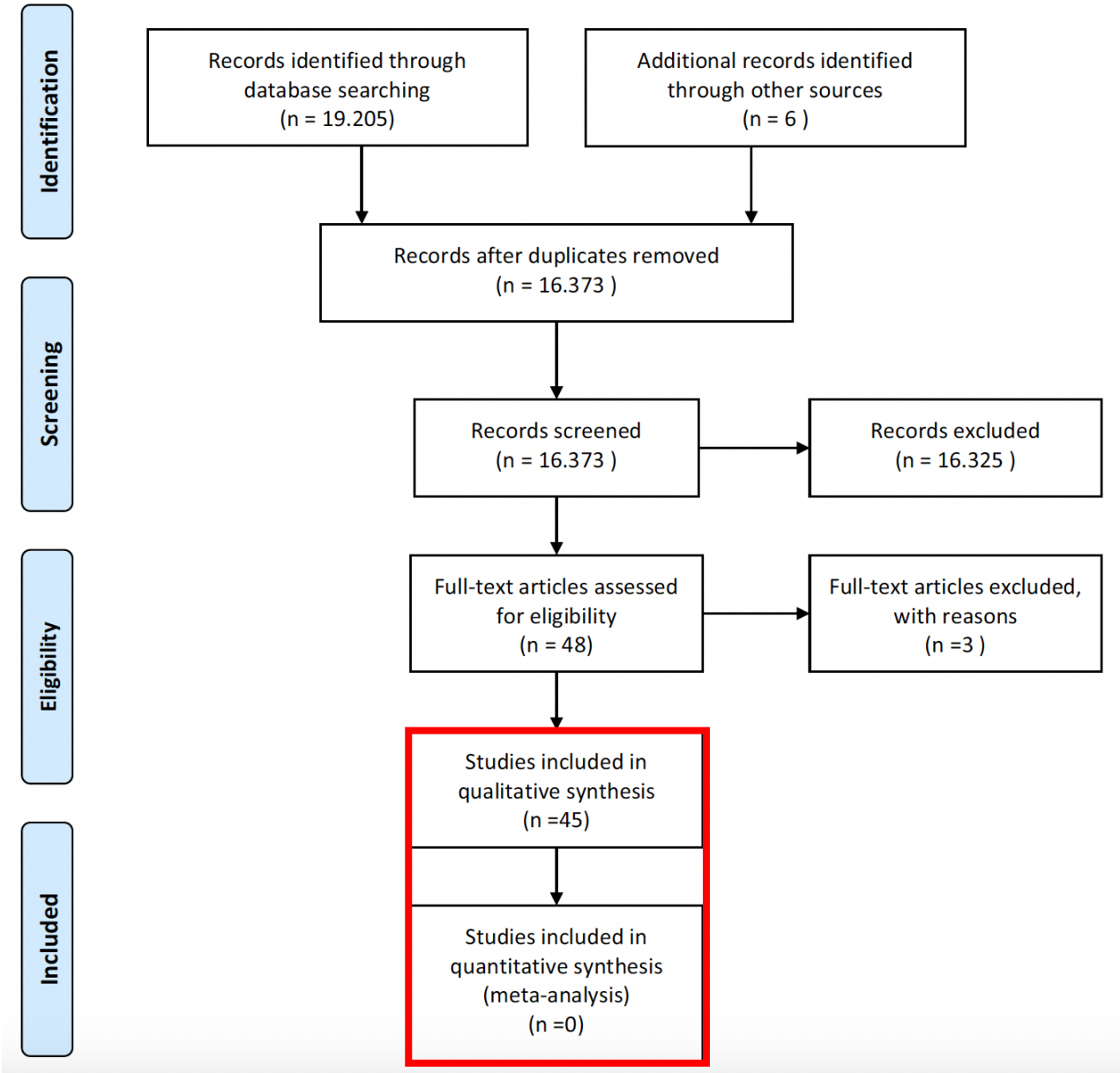
REVIEW ARTICLE

The use of ketogenic diets in cancer patients: a systematic review

Maximilian Römer[1] · Jennifer Dörfler[1] · Jutta Huebner[1]

Received: 9 February 2021 / Accepted: 26 March 2021 / Published online: 3 April 2021

Abstract
Ketogenic diets are a widely known, yet controversial treatment for cancer patients. In this review, we summarize the clinical evidence for anti-tumor effects, as well as the effects on anthropometry, quality of life, adverse events and adherence in cancer patients. In April 2019, a systematic search was conducted searching five electronic databases (EMBASE, Cochrane, PsychInfo, CINAHL and Medline) to find studies analyzing the use, effectiveness and potential harm of a ketogenic diet in cancer patients of any age as sole or complementary therapy. From all 19.211 search results, 46 publications concerning 39 studies with 770 patients were included in this systematic review. The therapy concepts included all forms of diets with reduced carbohydrate intake, that aimed to achieve ketosis for patients with different types of cancer. Most studies had a low quality, high risk of bias and were highly heterogeneous. There was no conclusive evidence for anti-tumor effects or improved OS. The majority of patients had significant weight loss and mild to moderate side effects. Adherence to the diet was rather low in most studies. Due to the very heterogeneous results and methodological limitations of the included studies, clinical evidence for the effectiveness of ketogenic diets in cancer patients is still lacking.



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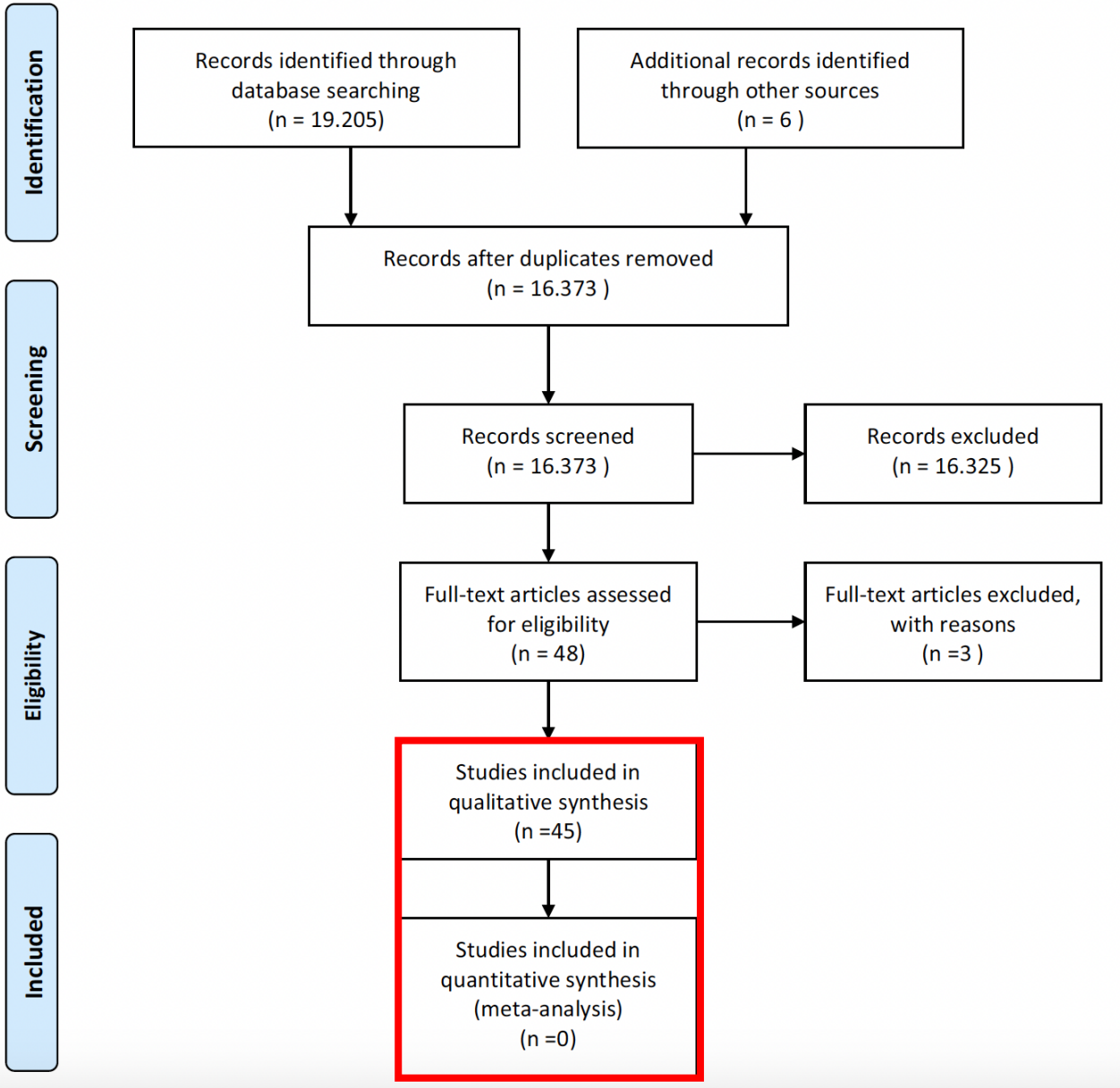

Nutrient supplementation approaches

- Ketogenic diets
- **Amino acid supplementation**
- **Unsaturated fatty acid supplementation**

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Saverio Minucci (IEO)

Giovanni Apolone (INT)

All the patients and their families!

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IFOM

Thank you for your attention!

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