Synergizing Multicellular Spheroids/Organoids, Microbial Natural Products, High Content Screening, and AI for Triple Negative Breast Cancer Therapy Discovery (SMART-TNT)

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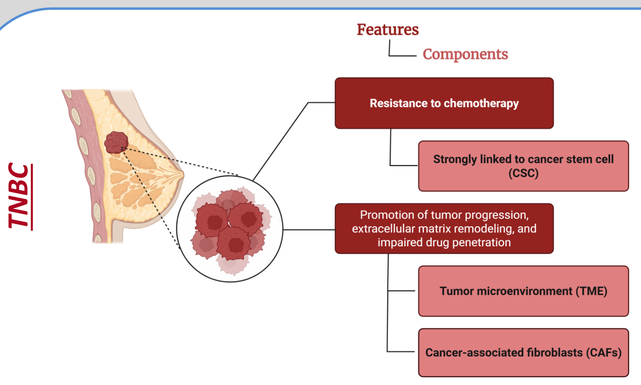
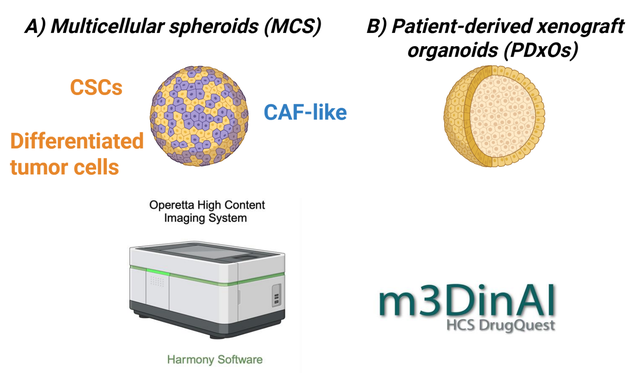
TNBC



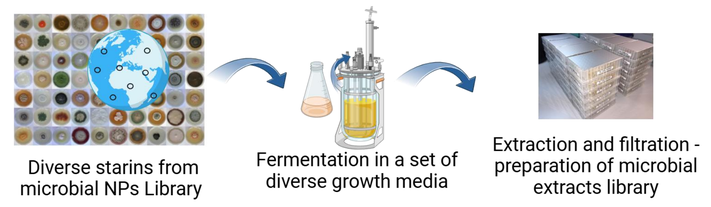
- **Triple-negative breast cancer (TNBC)** is an aggressive and highly heterogeneous cancer subtype which limits targeted therapeutic options and contributes to high mortality rates.

Drug discovery platform



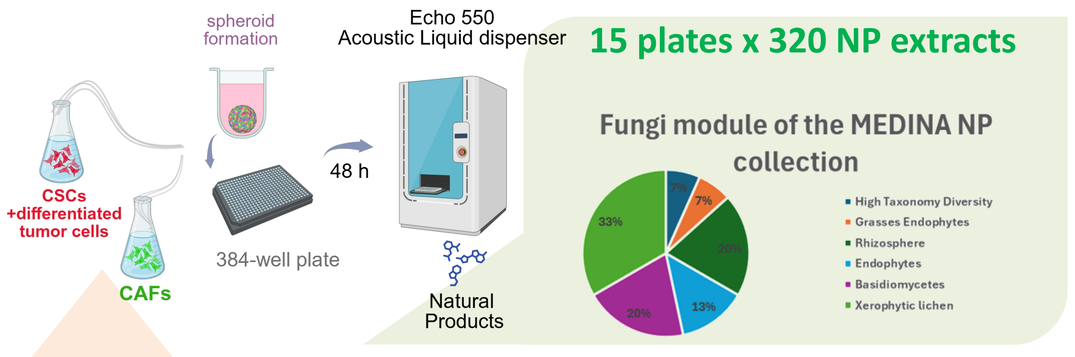
- The **SMART-TNT drug discovery platform** uses physiologically relevant 3D tumor models [1-2], high content imaging [3] and profiling analysis [4-5] to identify **new therapeutic strategies.**

MEDINA microbial NP extract library



- A subset of 4,800 **microbial natural products (NPs) extracts** with diverse origin from MEDINA's collection were screened for antitumor activity in this platform [3,6].

Multicellular spheroids (MCS) - Primary screening

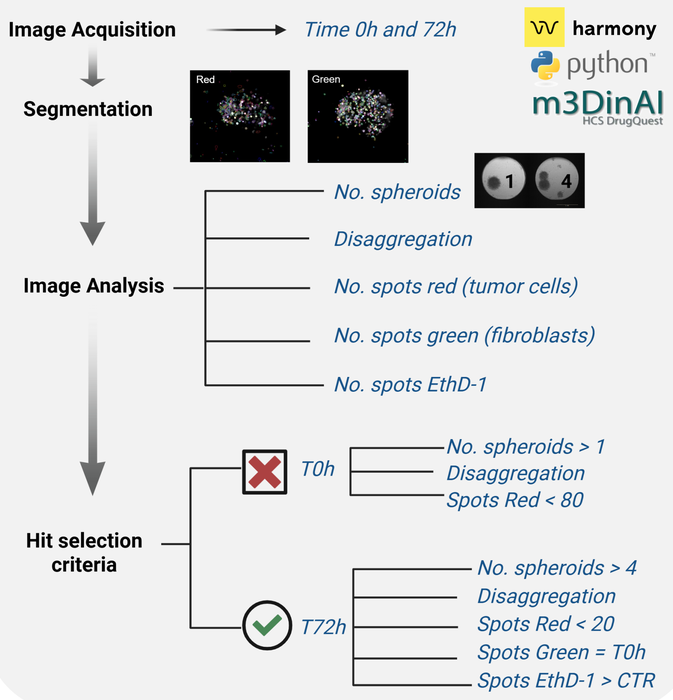


EthD-1 (after 72 h)

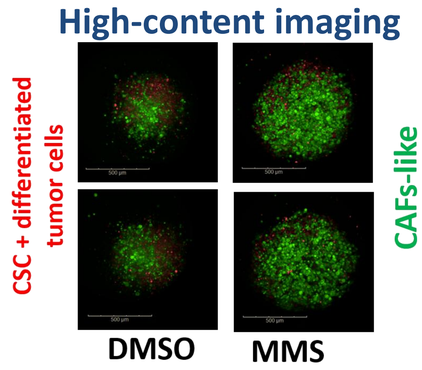
Operetta High Content Imaging System

Adquisition at T0 h and T72 h post-treatment

Image acquisition



High-content imaging

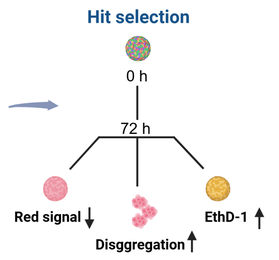


*DMSO: vehicle
* MMS: methyl methanesulfonate

130 extracts selected

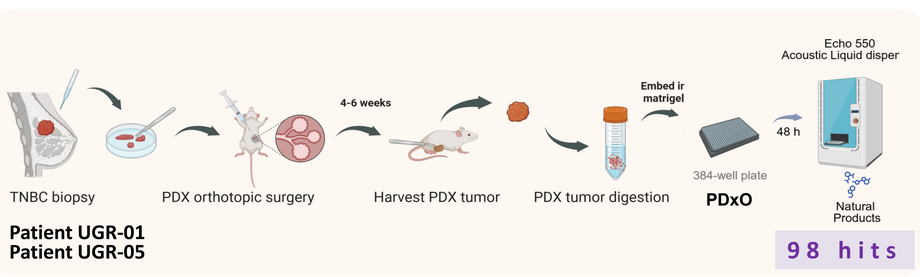
Cherrypicking

98 hits



Patient-derived xenograft organoids (PDxOs)- Extract confirmation

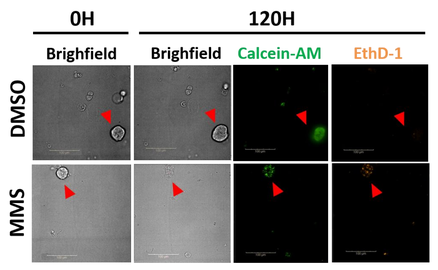
1. PDxO generation



Calcein
EthD-1
Hoechst
(after 120 h)

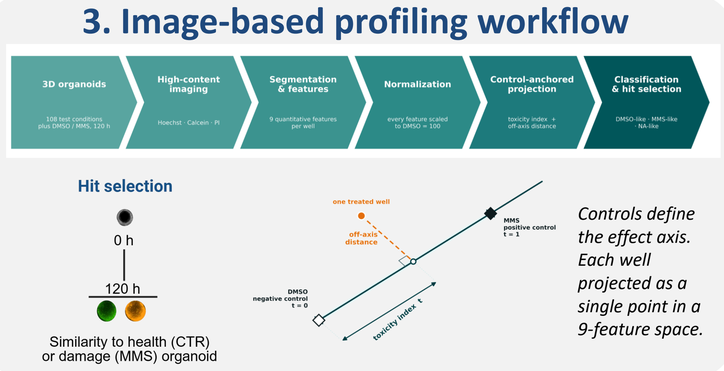
Operetta High Content Imaging System

2. Pilot High-content imaging



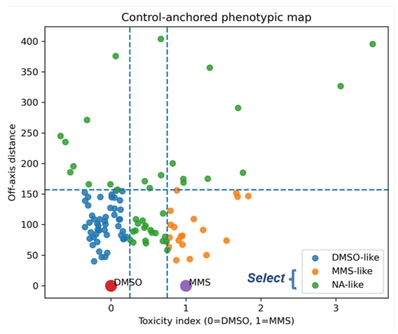
DMSO (C-), healthy organoids, calcein-AM+, EthD-1-
MMS (C+), damaged organoids, calcein-AM+/-, EthD+1-

3. Image-based profiling workflow



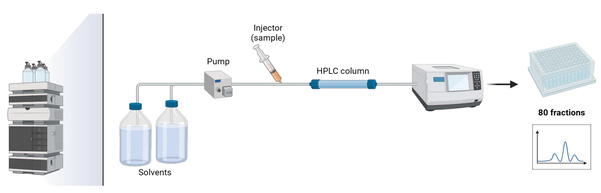
Controls define the effect axis. Each well projected as a single point in a 9-feature space.

4. Extract confirmation

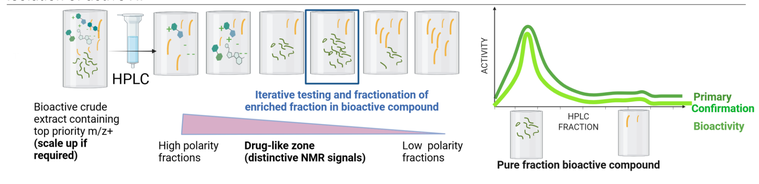


9 extracts selected and 9 excluded for toxicity in HepG2 cells

5. Polarity-based fractionation

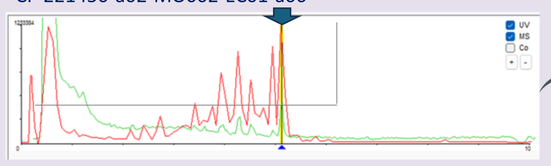


6. Fractions confirmation



7. Hit identification (2 fractions)

CF-221450-a02-MO002-EC01-a06



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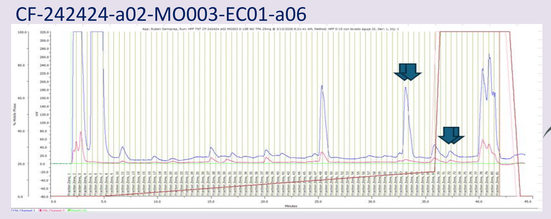
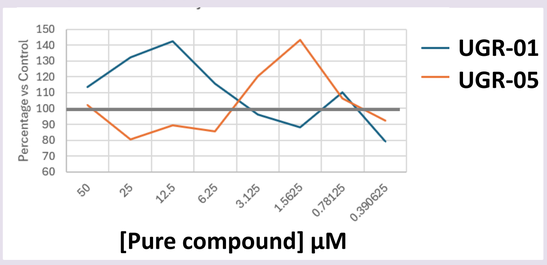


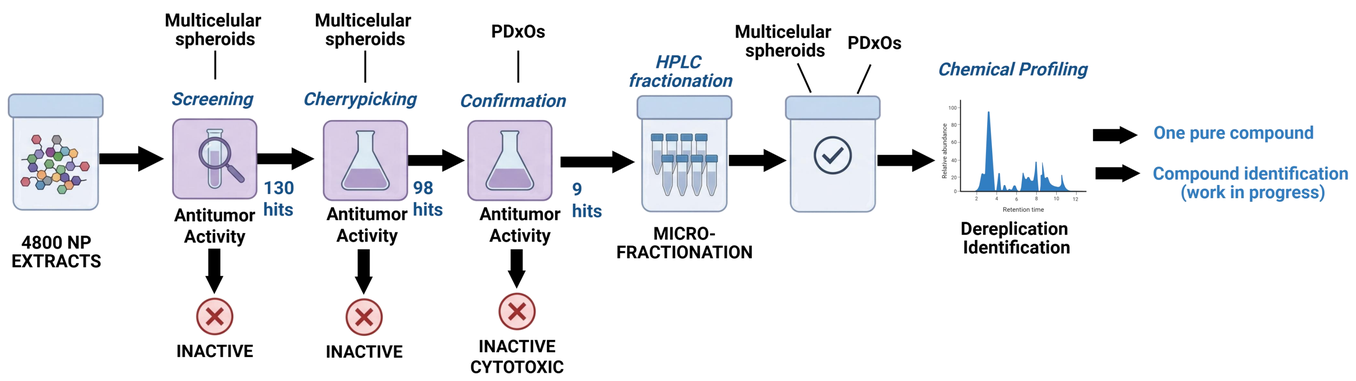
8. Hit validation (pure compound) on two PDxOs



Compound identification (work in progress)

SUMMARY & CONCLUSIONS

- ✓ The **SMART-TNT platform** successfully integrated MCS and PDxOs models with high-content imaging for NP-based drug discovery.
- ✓ **Proof of concept**: identification of candidate anti-tumor hits: **one pure compound and two active fractions.**



BIBLIOGRAPHY

1. **De Lara-Peña et al. 2024**. PMID: 38478342
2. **López-Tejada et al. 2025.** PMID: 40612674
3. **Mackenzie et al. 2024**. PMID: 38257340
4. **Iañez et al. 2026.** Preprint.doi.org/10.21203/rs.3.rs-9208673/v1
5. **Iañez et al. 2026.** Preprint.doi.org/10.2139/ssrn.7511696
6. **Ramos et al. 2024.** PMID: 38908208

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