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Exosome-Based Cancer Testing

Revolutionizing Early Cancer Detection by A Non-Invasive Saliva-Based Approach

THE EARLY DETECTION CRISIS

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2 mil New Cases

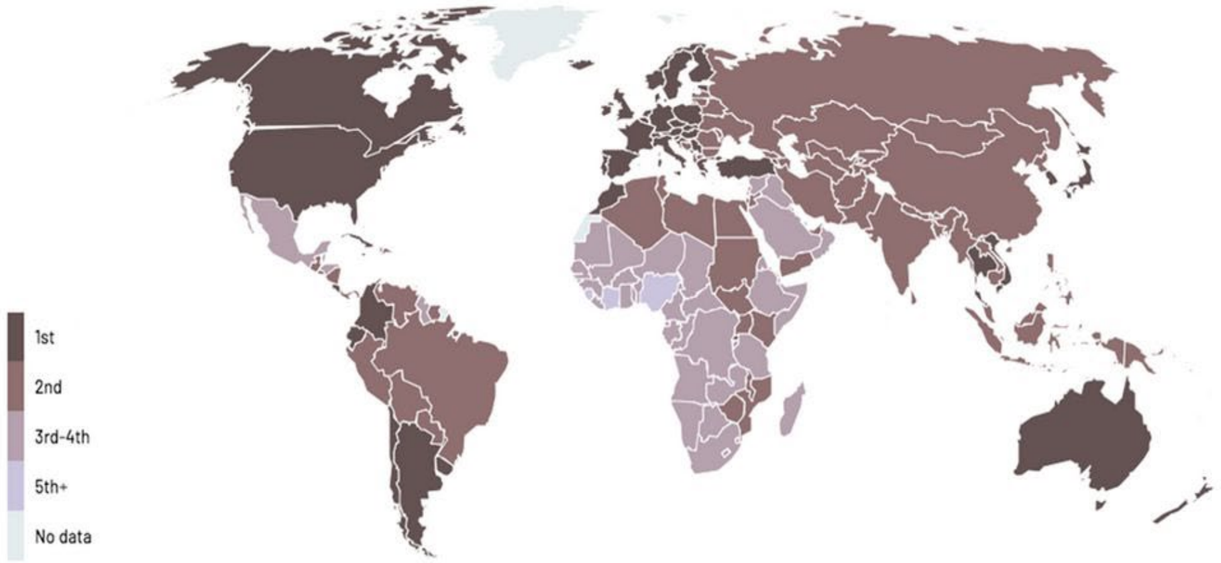
Cancer remains the #2 leading cause of death globally, with millions of new cases each year.

>60%

> 60% of cancers are diagnosed at late stages, when treatment options are limited.

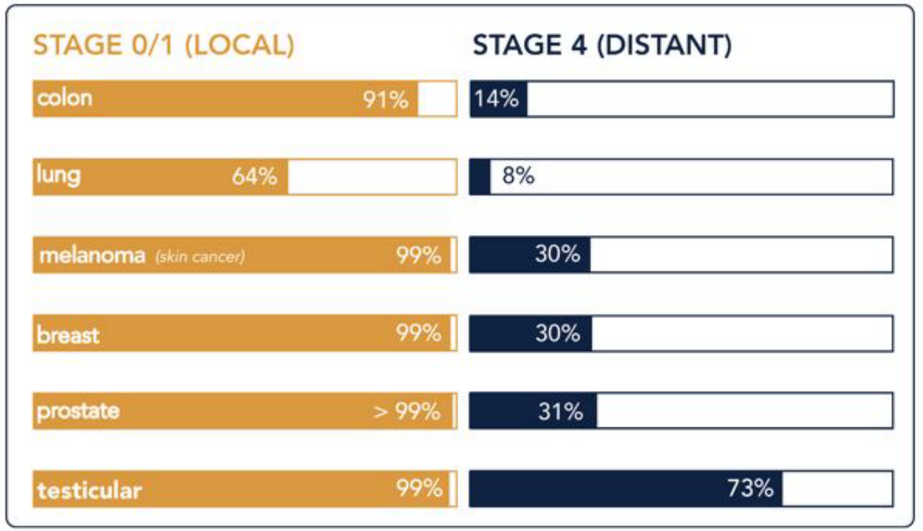
- Early detection can double or triple survival rates in many cancer types.
- Current screening methods fail to detect cancer at its earliest, most treatable stages.
- There is a critical global demand for sensitive, non-invasive, and scalable early detection solutions.

Cancer ranks as the leading cause of death among 30-69



World Health Organization. Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000–2016. 2018.

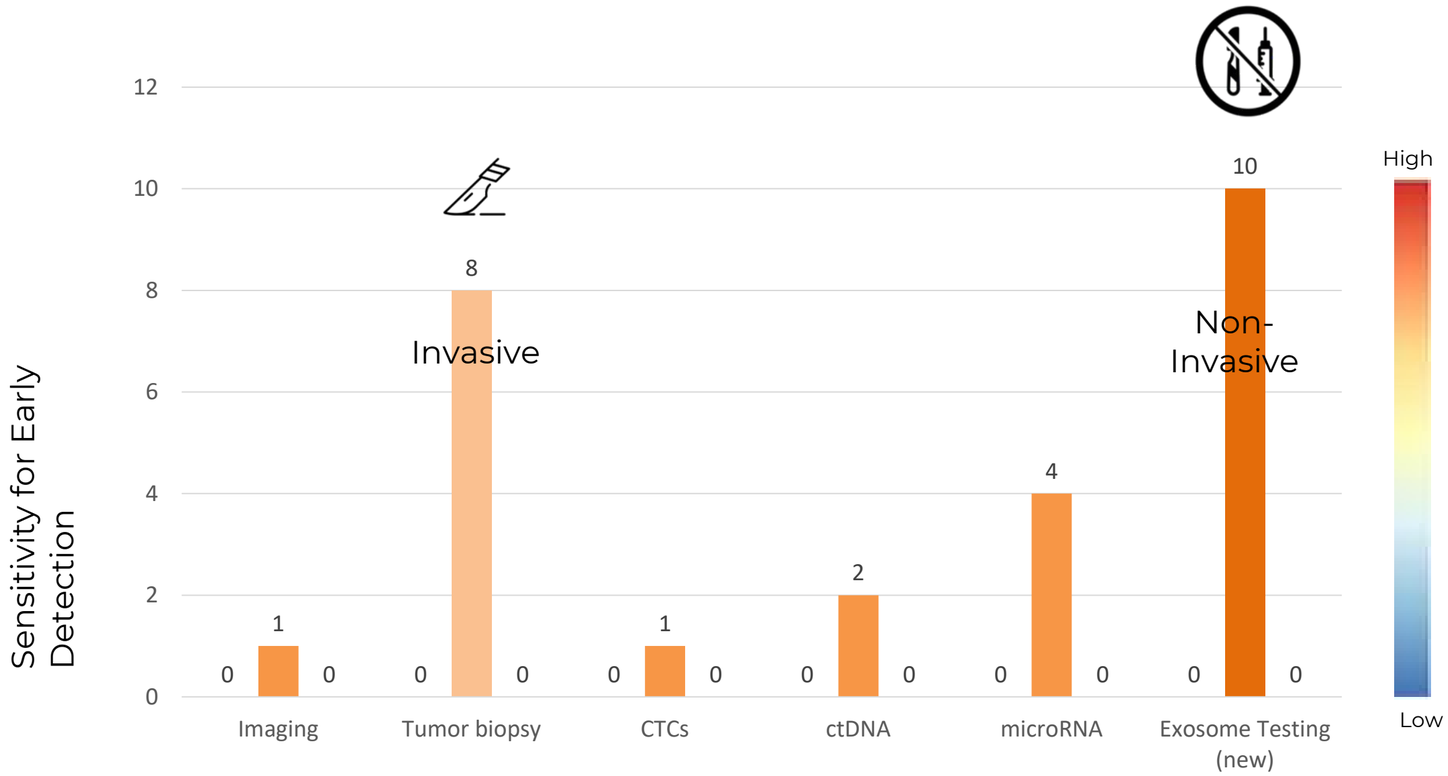
Early vs. Late Diagnosis Survival Rates



SOURCE: American Cancer Society 5-year SEER data (based on people diagnosed between 2011-2017)

The Early Detection Gap: Current Technologies vs. Early-Stage Cancer

- IMAGING (MRI, PET, CT)
- TISSUE BIOPSY
- CIRCULATING TUMOR CELLS (CTCS)
- CIRCULATING TUMOR DNA (CTDNA)
- MICRO RNA
- EXOSOME TESTING (NEW)



Current methods miss early cancers.
Non-invasive exosome tests bridge the gap.

Simplest Solution in Early Cancer Testing

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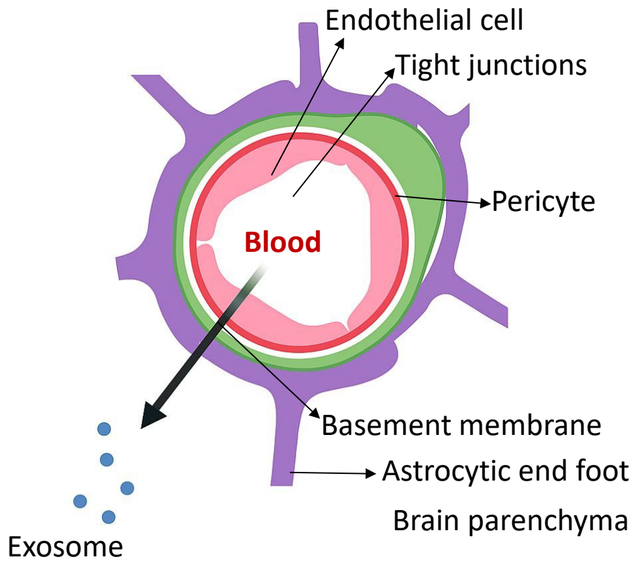
SALIVA TEST KIT

COST EFFECTIVE

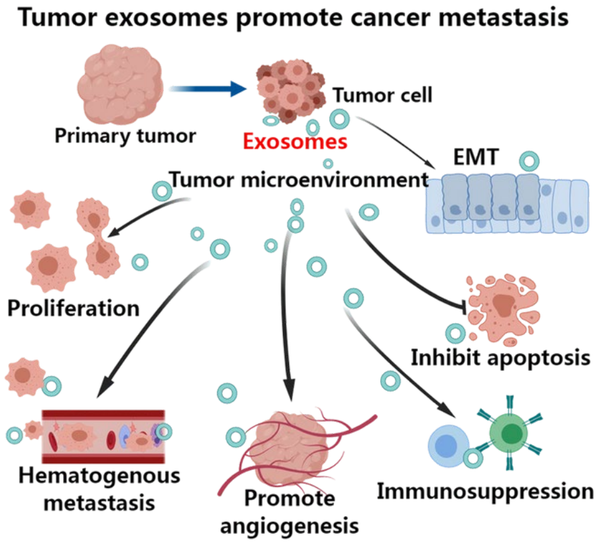
ACCURATE

FAST

Making Worldwide Universal Early Screening a Reality



Blood-brain barrier



Why Exosomes?

Nature's Cancer Messengers

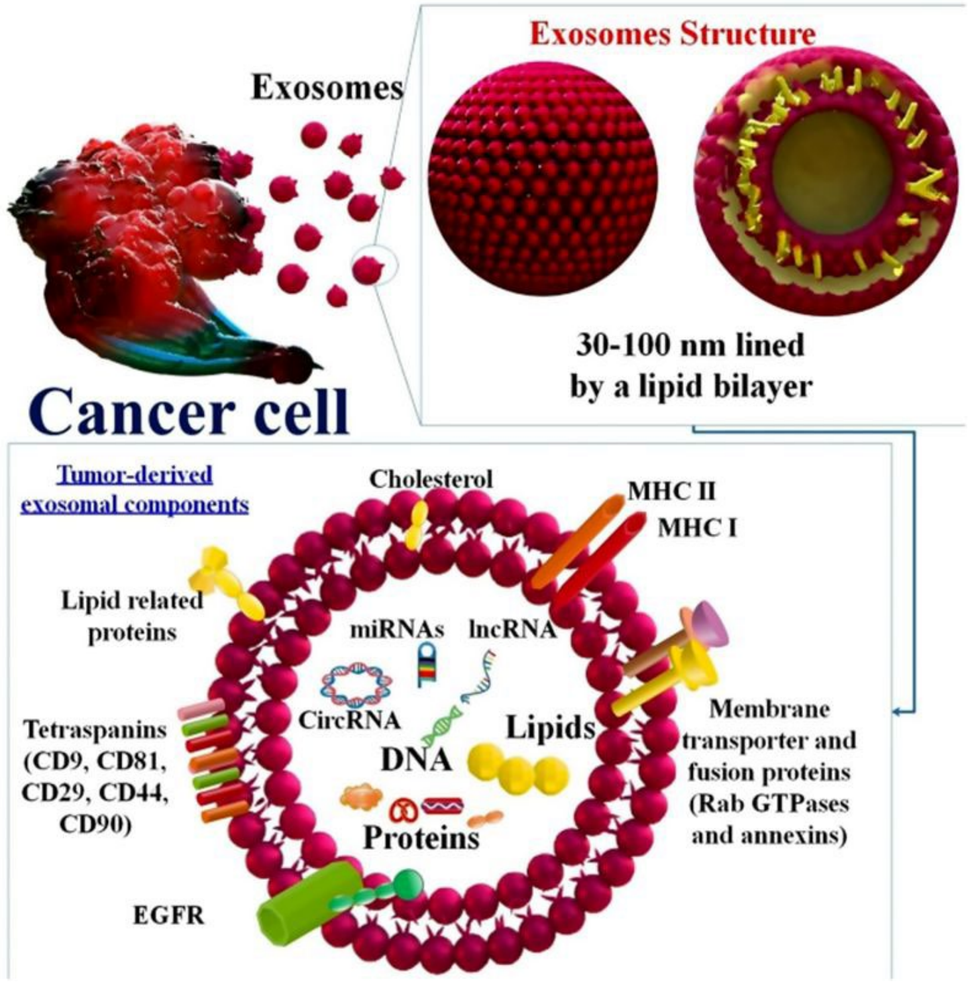
- **Nanoscale vesicles** naturally secreted by tumor cells, protecting molecular cargo **(DNA, RNA, proteins)** during circulation .
- Analysis of their cargo directly reveals **tumor biology** and their surface membrane reveals **tumor origin.**
- Capable of **crossing biological barriers** (e.g., blood-brain barrier), and distributed systemically throughout the body.
- Enable detection of distant, hidden, or difficult-to-access **tumors through non-invasive sampling** (e.g., saliva-based testing).
- Provide real-time monitoring of **tumor progression** and **therapeutic response.**
- Allow detection even at **early-stage or minimal disease** burden.

https://www.researchgate.net/figure/Tumor-derived-exosomes-promote-cancer-metastasis-Tumor-derived-exosomes-through-multiple_fig1_354552712

Tumor-Derived Exosomes

Exosomes have been cruising under the radar for a long time because they are small and there are many other circulating vesicles of similar sizes with similar structures, plus they've always been considered cellular waste. But they are now having their moment, due to their potential to complement or even outperform other circulating biomarkers such as circulating tumor cell (CTC), circulating tumor DNA (CTDNA), and traditional circulating protein biomarkers, which also aim to survey the tumor information from the blood.

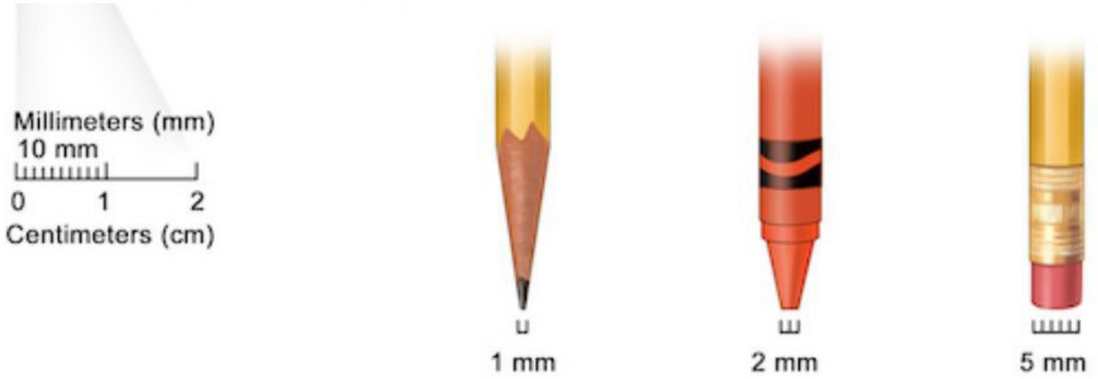
Poised to outperform current liquid biopsy markers



https://cancerci.biomedcentral.com/articles/10.1186/s12935-024-03379-1

Finding Cancer at the Cellular Stage

Detection at this level is possible using exosomes



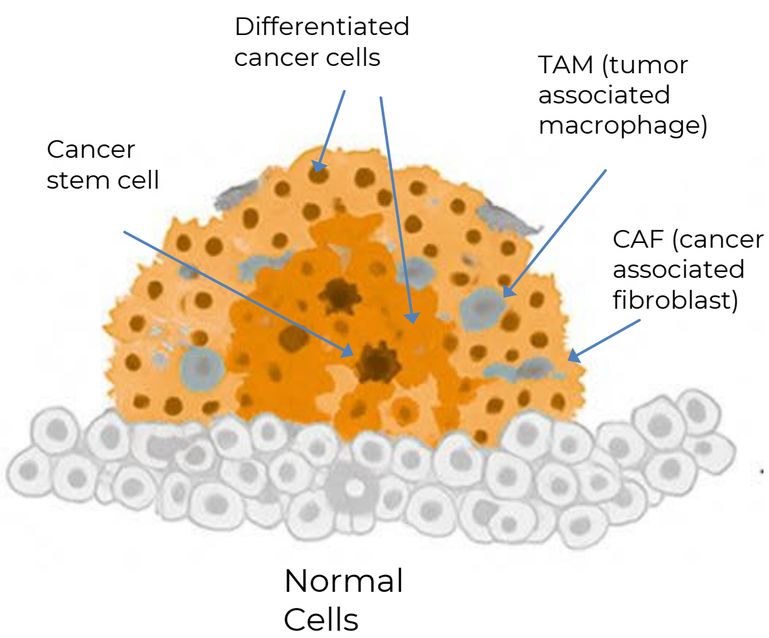
1
The Ultimate Goal of Cancer Testing

2
Finding them when they are small and haven’t spread.

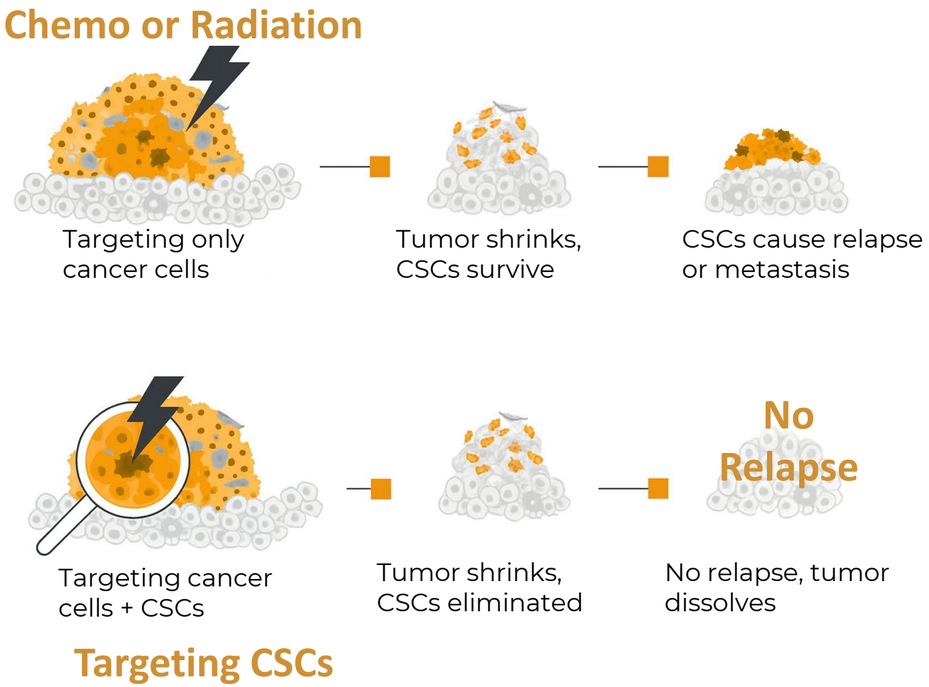
3
Closing the early detection gap with high sensitivity testing technology

https://www.rogelcancercenter.org/research/stem-cells

The Hidden Drivers of Cancer Aggression



Normal Cells



Cancer Stem Cells (CSCs)

- Hallmark of Aggressive Cancer
- CSCs are only (1-3%) of a tumor but very aggressive

https://www.rogelcancercenter.org/research/stem-cells

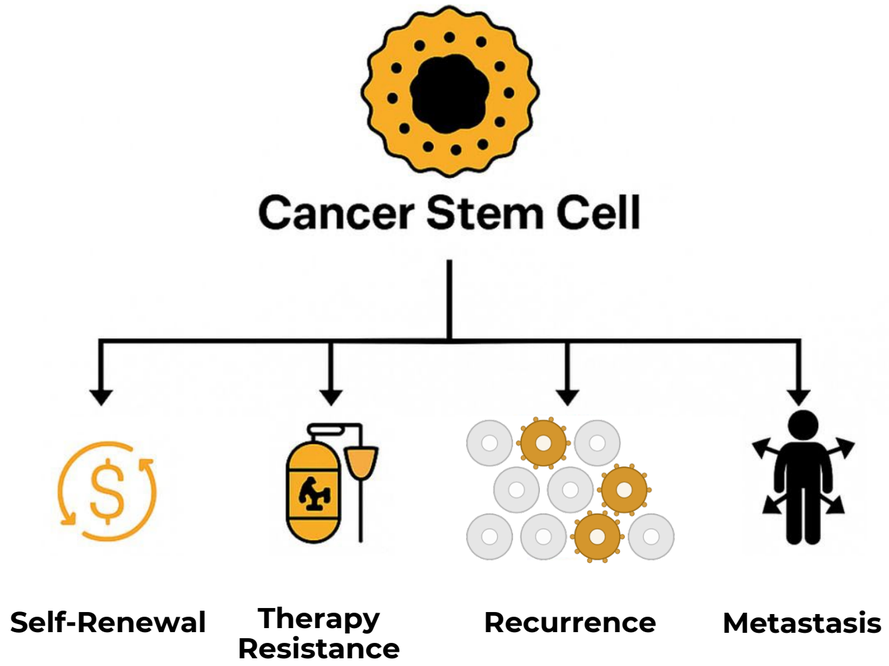
Features of Cancer Stem Cells

SELF-RENEWAL: SUSTAIN CONTINUOUS TUMOR GROWTH.

TUMOR RECURRENCE: REGENERATE TUMORS EVEN AFTER INITIAL TREATMENT RESPONSE.

THERAPY RESISTANCE: SURVIVE CHEMOTHERAPY, RADIATION, AND TARGETED THERAPIES.

METASTATIC POTENTIAL: DRIVE SPREAD TO DISTANT ORGANS.



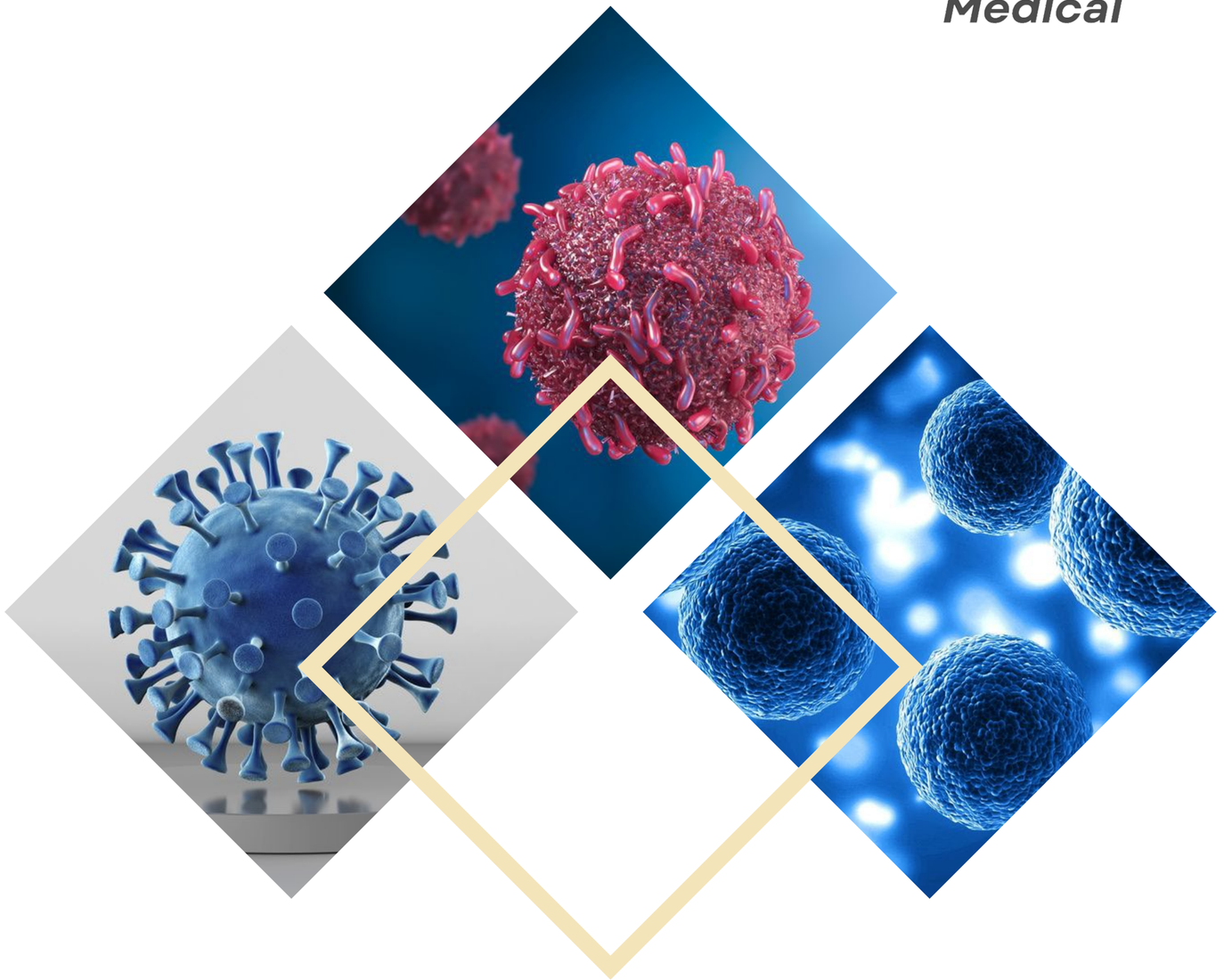
CSCs are regulated by pluripotency genes such as **NANOG, OCT4, and SOX2**. **Detecting CSC-specific markers offers a powerful strategy to identify the most aggressive, treatment-resistant cancers — even at early stages.**

NANOG expression in
Cancer Stem Cells (CSC)

NANOG gene family as a diagnostic marker in cancer

Background

- NANOG: Embryonic transcription factor crucial for stem cell pluripotency
- NANOGP8: Pseudogene variant associated with cancer stem cell (CSC) phenotype
- Exosomes: vessels that contain fragments of cancerous DNA



NANOG: MASTER SWITCH IN CANCER STEM CELLS

1

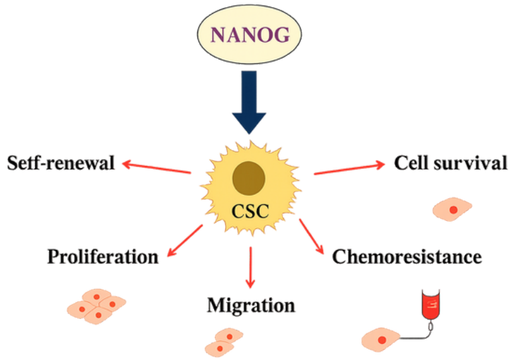
NANOG is a master regulator of pluripotency, essential for maintaining stem cell-like behavior in cells.

2

Abnormally activated in CSCs, particularly within cells that drive cancer growth, progression, metastasis, and treatment resistance.

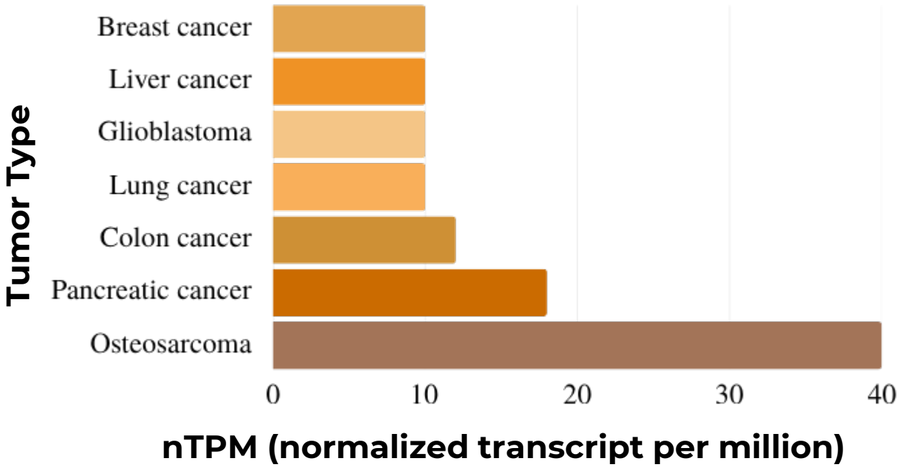
3

Elevated NANOG expression correlates with poor prognosis and unfavorable clinical outcomes across multiple tumor types.



11

NANOG GENE EXPRESSION BY TUMOR TYPE



HIGHLY ASSOCIATED WITH AGGRESSIVE, TREATMENT-RESISTANT CANCERS, INCLUDING:

- **Glioblastoma (GBM)**
- **Pancreatic cancer**
- **Breast cancer**
- **Lung cancer**
- **Colon cancer**

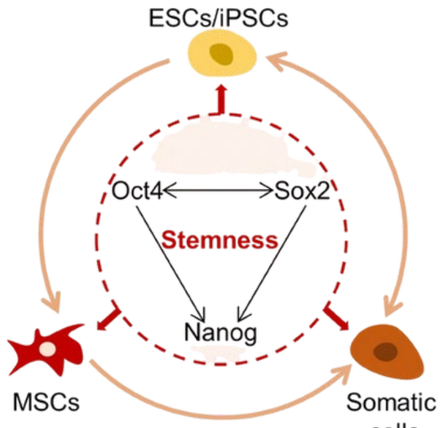
https://pmc.ncbi.nlm.nih.gov/articles/PMC6134963/

What is NANOG

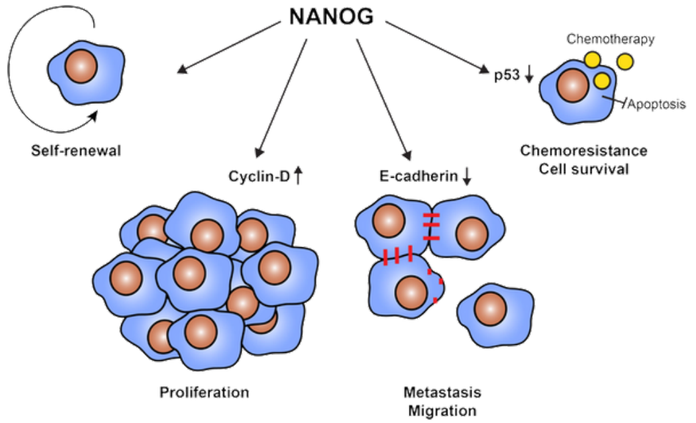
Core **EMBRYONIC GENE** together with Oct4 and Sox2 that maintains the pluripotent state of embryonic stem cells (ESCs) which can differentiate into any cell type.

Typically, only found in humans during the **embryonic stage** of life (10 weeks) or in **Cancer Stem Cells (CSCs)**

Aberrantly re-expressed in CSCs across **various tumor types**



https://link.springer.com/article/10.1007/s12015-021-10154-6



https://www.researchgate.net/publication/325853003_Immune_Curbing_of_Cancer_Stem_Cells_by_CTLs_Directed_to_NANOG

Differentiating Between

Cancerous & Normal

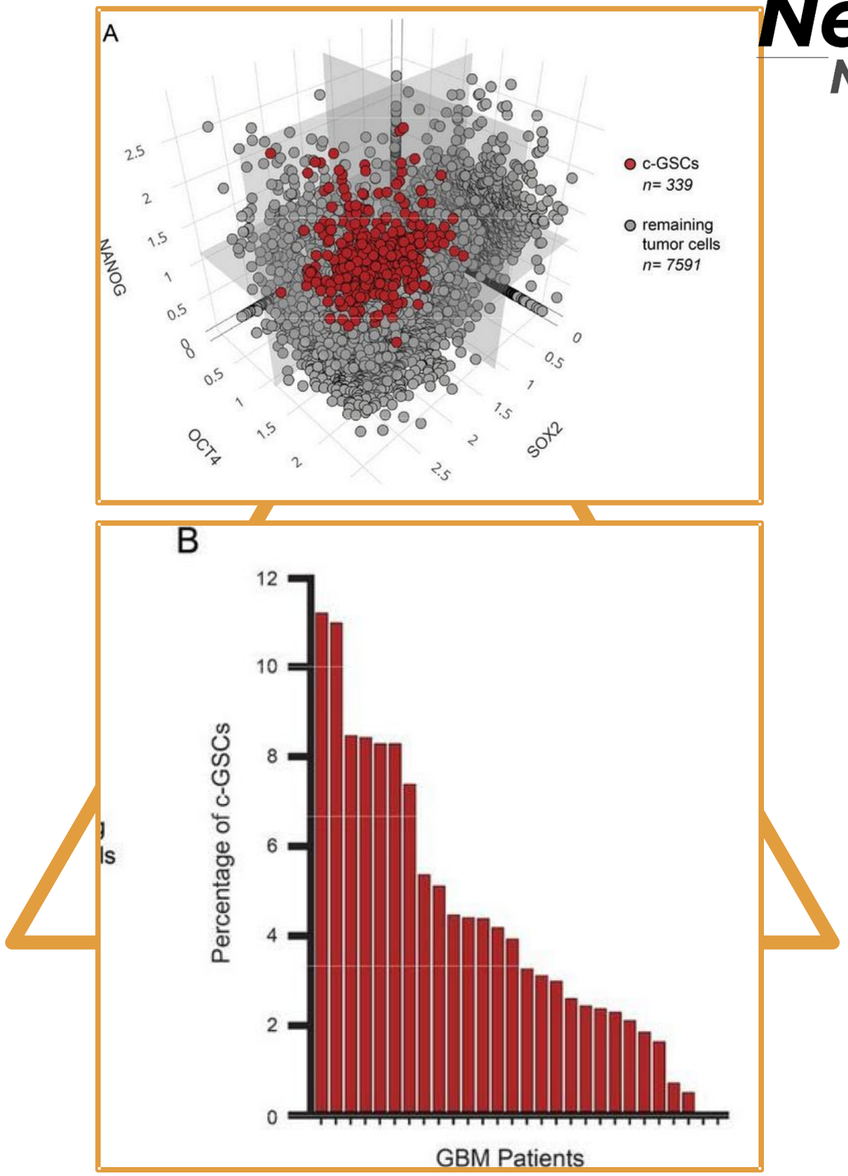
Can exosomal **NANGOG DNA** sequences differentiate cancerous from non-cancerous cells?

Core Glioma Stem Cells (c-GSCs) have 3 genes – NANOG, OCT4, SOX2

92.8% of GBM patients have c-GSCs w/ average presence of 4.22%

(A) Three-dimensional scatter plot of GBM single-cells (n = 7,930) from 28 patients based on the expression levels of OCT4 (x-axis), NANOG (y-axis), and SOX2 (z-axis). Red dots represent c-GSCs co-expressing OCT4, SOX2, and NANOG (n = 339). Gray dots represent the remaining cells from tumor bulk (n = 7,591).
(B) Bar plot showing the percentage of c-GSCs in each GBM patient.

NANOG – A promising biomarker differentiating cancer stem cells from normal cells



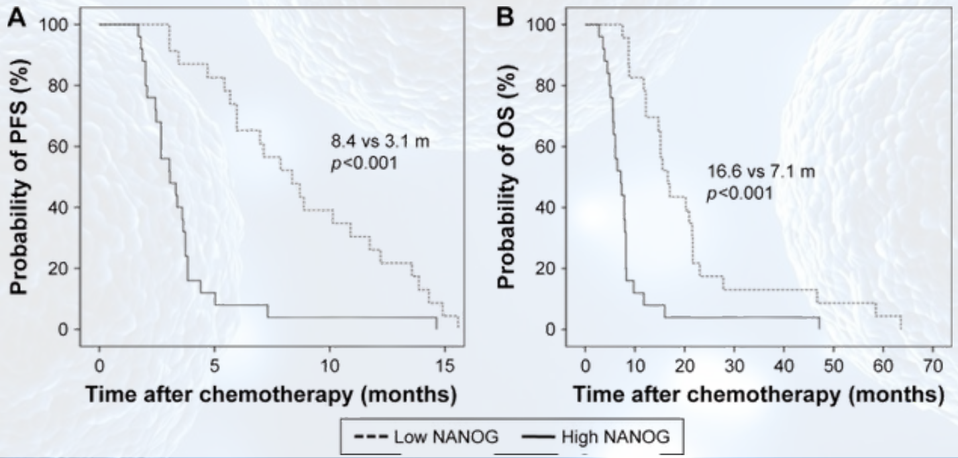
https://www.biorxiv.org/content/10.1101/2021.12.07.471556v1.full

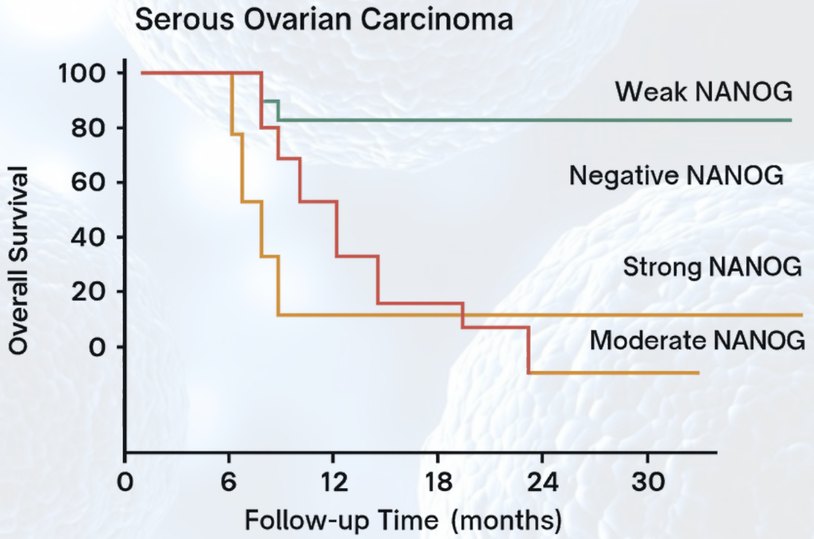
Ideal Cancer Biomarker

NANOG Overexpression in Cancer Stem Cells (CSCs)

1. Marker of aggressive forms of cancer (GBM, Pancreatic, Breast, Lung, Colon)
2. NANOG expression correlated with poor prognosis in cancers

Squamous Cell Carcinoma





https://www.sciencedirect.com/science/article/abs/pii/S0378111922002670
https://onlinelibrary.wiley.com/doi/10.1155/2016/7028289
https://pubmed.ncbi.nlm.nih.gov/29033581/

GLIOBLASTOMA AND CANCER STEM CELLS (CSCS)

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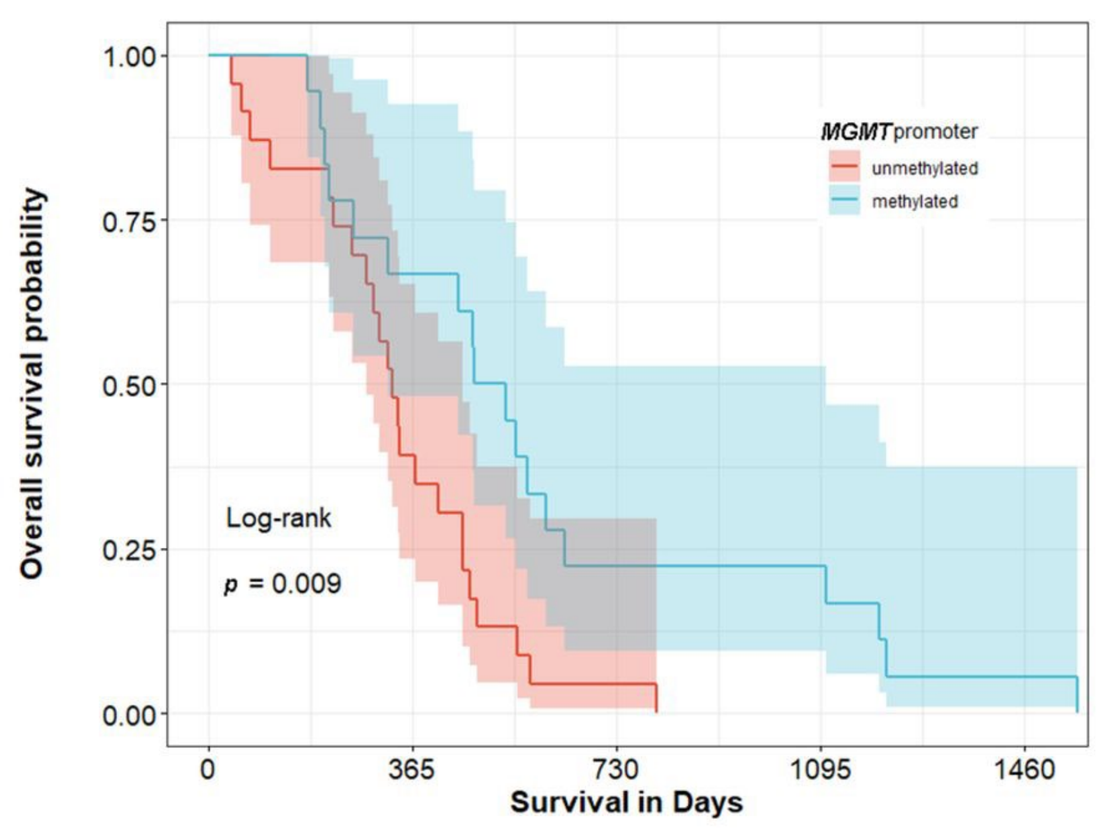
Glioblastoma



- ❑ **GBM: Lethal & therapy-resistant**
- ❑ **CSC markers = early detection**

Most Aggressive

Glioblastoma (GBM) is the most aggressive and lethal brain cancer.

Low Survival

Median survival is only 12–15 months despite advanced treatment.

Therapy Resistance

It's highly invasive and resistant to standard therapies.

Tumor Regrowth

Unmethylated patients have resistant cells that often survive and regenerate the tumor.

CSC Markers

NANOG, OCT4, and SOX2 help identify aggressive tumors early.

PROOF OF CONCEPT: EXOSOMAL NANOG DNA DETECTS CANCER

Objective:

Identify exosome-associated NANOG DNA variations as diagnostic markers for cancer.

Methods

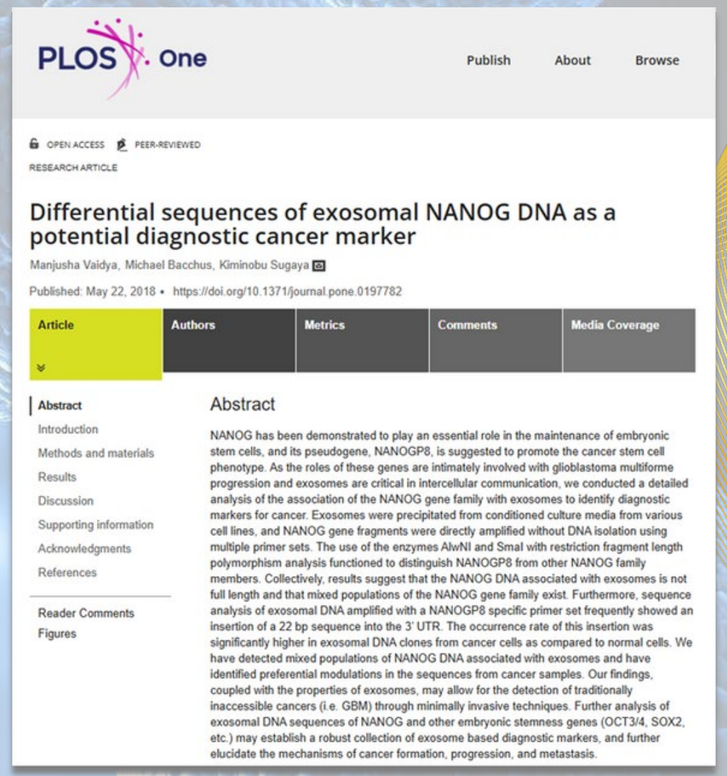
- Exosomes isolated from cancer and normal cell lines (GBM, breast, lung, etc.).
- Direct PCR amplification of exosomal DNA
  - No complex DNA extraction needed.
  - Minimal sample prep.
  - Rapid turnaround time.
  - Cost-effective.

Key Findings:

- Exosomal DNA contained fragmented NANOG/NANOGP8 sequences.
- Cancer-derived exosomes showed a significantly higher frequency of a unique 22 bp insertion in NANOGP8 3'UTR.
- Distinct sequence patterns differentiate cancer from non-cancer cells.

Implications:

- Exosomal NANOG DNA reflects cancer-specific genomic alterations.
- Demonstrates high specificity for malignant cells.
- Consistent detection across multiple cancer types.



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RESEARCH ARTICLE

Differential sequences of exosomal NANOG DNA as a potential diagnostic cancer marker

Manjusha Vaidya, Michael Bacchus, Kiminobu Sugaya

Published: May 22, 2018 • https://doi.org/10.1371/journal.pone.0197782

Article | Authors | Metrics | Comments | Media Coverage

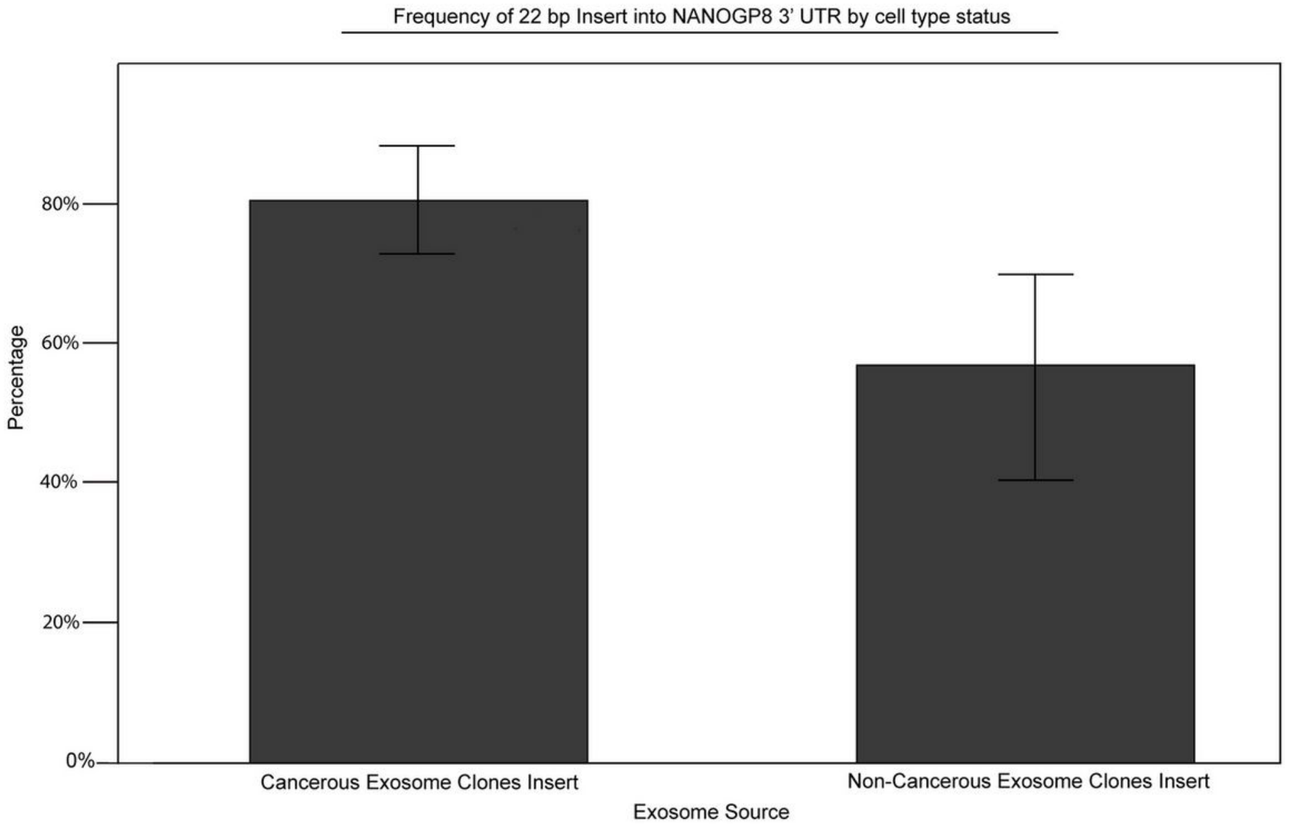
Abstract
Introduction
Methods and materials
Results
Discussion
Supporting information
Acknowledgments
References
Reader Comments
Figures

Abstract

NANOG has been demonstrated to play an essential role in the maintenance of embryonic stem cells, and its pseudogene, NANOGP8, is suggested to promote the cancer stem cell phenotype. As the roles of these genes are intimately involved with glioblastoma multiforme progression and exosomes are critical in intercellular communication, we conducted a detailed analysis of the association of the NANOG gene family with exosomes to identify diagnostic markers for cancer. Exosomes were precipitated from conditioned culture media from various cell lines, and NANOG gene fragments were directly amplified without DNA isolation using multiple primer sets. The use of the enzymes AlwNI and SmaI with restriction fragment length polymorphism analysis functioned to distinguish NANOGP8 from other NANOG family members. Collectively, results suggest that the NANOG DNA associated with exosomes is not full length and that mixed populations of the NANOG gene family exist. Furthermore, sequence analysis of exosomal DNA amplified with a NANOGP8 specific primer set frequently showed an insertion of a 22 bp sequence into the 3' UTR. The occurrence rate of this insertion was significantly higher in exosomal DNA clones from cancer cells as compared to normal cells. We have detected mixed populations of NANOG DNA associated with exosomes and have identified preferential modulations in the sequences from cancer samples. Our findings, coupled with the properties of exosomes, may allow for the detection of traditionally inaccessible cancers (i.e. GBM) through minimally invasive techniques. Further analysis of exosomal DNA sequences of NANOG and other embryonic stemness genes (OCT3/4, SOX2, etc.) may establish a robust collection of exosome based diagnostic markers, and further elucidate the mechanisms of cancer formation, progression, and metastasis.

https://cancerci.biomedcentral.com/articles/10.1186/s12935-024-03464-5

Key Findings



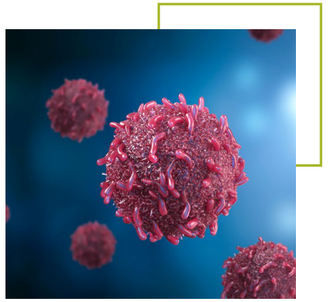
Exosomal DNA characteristics

- NANOG DNA is not full-length
- Mixed populations of NANOG family members detected

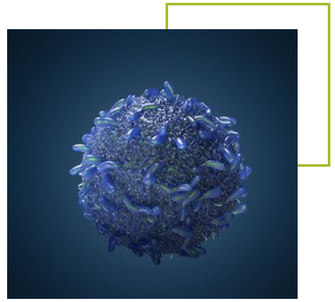
Cancer-Specific Patterns

- NANOG 22 base pair sequences detected in cancer cells
- Less consistent in non-cancer exosomes

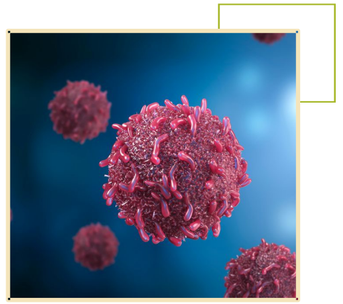
Development of a Method



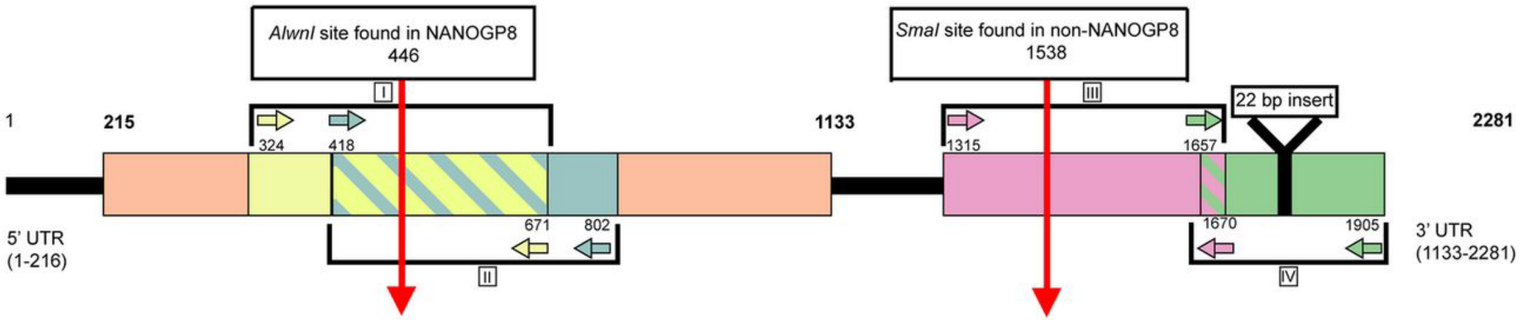
Sample collection of exosomes from various cancerous and non-cancerous cell lines



Technique
- Direct amplification of NANOG DNA (no isolation)
- Multiple primer sets utilized
- RFLP analysis w/AlwNI and SmaI enzymes to distinguish NANOGP8



Advantages of Methodology
Rapid, minimal sample prep



Primers and their locations on the NANOG/NANOGP8 gene:
I: 324-671 : NANOGP8-AlwNI-F1/R1 (347 bp) -> (5' AAAGCTTGCCTTGCTTTGAA 3') / (5' TCTGCTGGAGGCTGAGGTAT 3')
II: 418-802: NANOGP8-AlwNI-F2/R2 (384 bp) -> (5'-GTCTTCTGCTGAGATGCCTCACA-3') / (5'-CTTCTGCGTCACACCATTGCTAT-3')
III: 1315-1670: NANOG-SmaI-3'-UTR-F1/R1 (355 bp) -> (5'-AACCACGTGTTCTGGTTTCC-3') / (5'-GATCGAGACCATCCTGGCTA-3')
IV: 1657-1905: NANOGP8-3'-UTR-F2/R2 (248 bp) -> (5' GGATGGTCTCGATCTCCTGA 3') / (5' CCCAATCCCAAACAATACGA 3')

Exosome Saliva Testing

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1

Isolation of Exosomes from culture (60 min)

- PEG –NaCl precipitation
- Incubation overnight 4 degrees C
- Supernat centrifuged for 60 min @10G

2

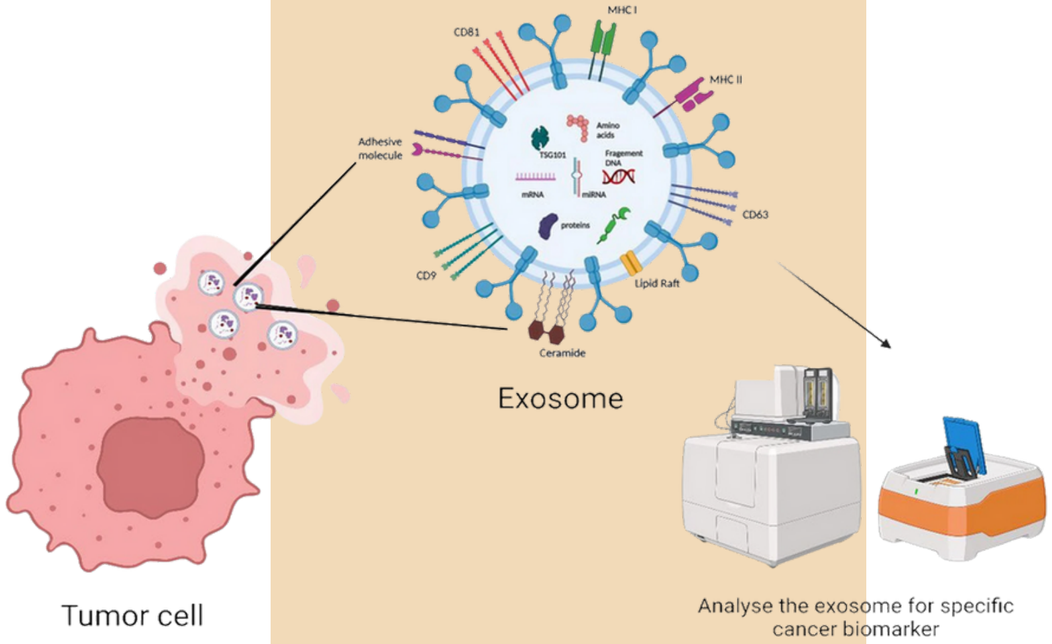
PCR Setting and Electrophoresis

3

Neutralization of Saliva Enzymes

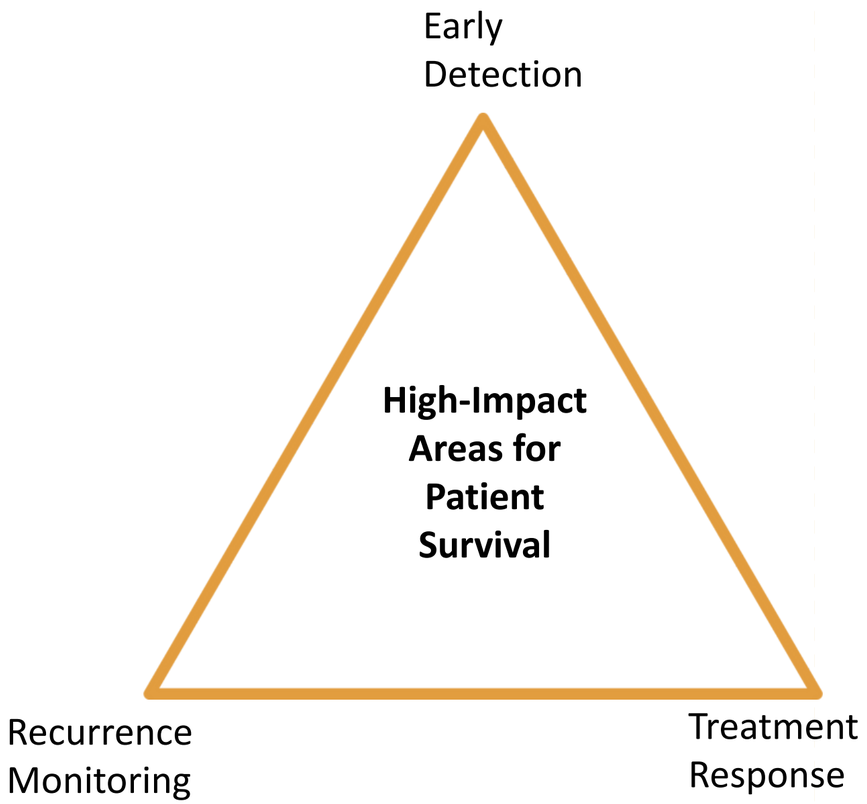
4

Primer designed to target NANOG & NANOGP8



https://cancerci.biomedcentral.com/articles/10.1186/s12935-024-03464-5

Significant Unmet Medical Needs Remain



MONITORING FOR RECURRENCE

Relapses because we cannot detect residual disease early

EARLY-STAGE DETECTION GAP

Over 60% of cancers are diagnosed late when survival is lowest

THERAPY RESPONSE TRACKING

Lack of fast, repeatable, non-invasive tests limits personalized treatment adjustments

$13B+ Market Ready for Disruption

Non-Invasive Focus

100% 10%

Liquid biopsy enables minimal discomfort

Projected Surge

2030 -33%

Rapid adoption curve expected by this year

Scalable platform

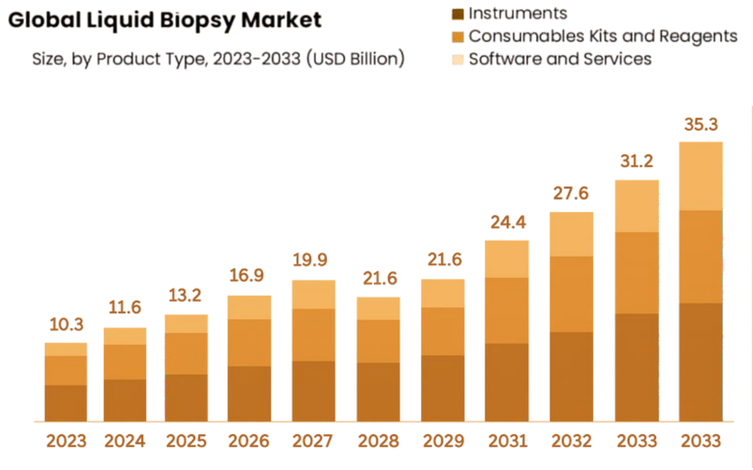
Broad adoption for population-wide screening & monitoring

Strong partnership potential

With diagnostics, pharma, and clinical labs

Significant unmet needs remain

- **Early-stage detection** — where intervention has the greatest impact.
- **Monitoring recurrence** — enabling proactive, ongoing surveillance.
- **Therapy response tracking** — guiding personalized treatment decisions.



Liquid biopsy market projected to surpass **$24B by 2030**, driven by demand for non-invasive cancer diagnostics.

https://www.globenewswire.com/news-release/2024/01/24/2814915/0/en/Liquid-Biopsy-Market-Size-of-USD-35-3-Billion-Anticipated-by-2033-Robust-CAGR-of-13-1.html

Development Roadmap

Phase I:

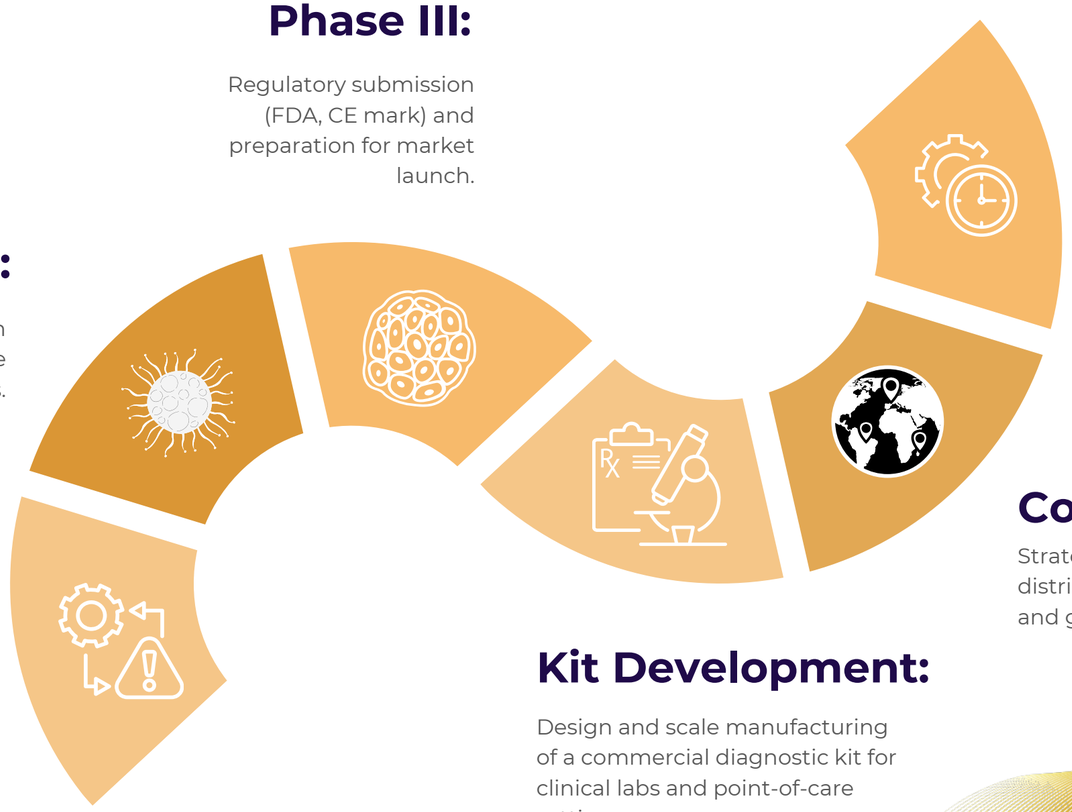
Clinical validation with cancer patient samples to establish diagnostic performance.

Phase II:

Multi-cancer expansion studies across diverse populations.

Phase III:

Regulatory submission (FDA, CE mark) and preparation for market launch.



Kit Development:

Design and scale manufacturing of a commercial diagnostic kit for clinical labs and point-of-care settings.

Commercialization:

Strategic partnerships for distribution, market penetration, and global scaling.

Long-term vision:

Establish platform as a routine, non-invasive screening tool for broad population health management.

Development Timeline

	Validation		Expansion		Regulatory (FDA)		Commercialization
# Months	0	6	12	18	24	30	36+
	Biomarker ID	Design & usability testing	Clinical validation studies	Clinical data collection	Manufacturing scale-up	Market launch Distribution Sales channels Post-market surveillance	Labeling

Collaboration or Investment Opportunity

First-in-class, saliva-based exosomal DNA diagnostic platform targeting early cancer detection.

Backed by strong preclinical proof-of-concept data demonstrating clinical feasibility.

Patent-pending proprietary technology with broad multi-cancer applicability.

Attractive near-term milestones and long-term scalability.

Seeking strategic collaboration or investment to accelerate:

- Clinical trials and multi-cancer validation
- Regulatory approvals (FDA, CE mark)
- Diagnostic kit development and manufacturing
- Commercial partnerships and global market expansion

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Thank You

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http://www.nextelmedical.com