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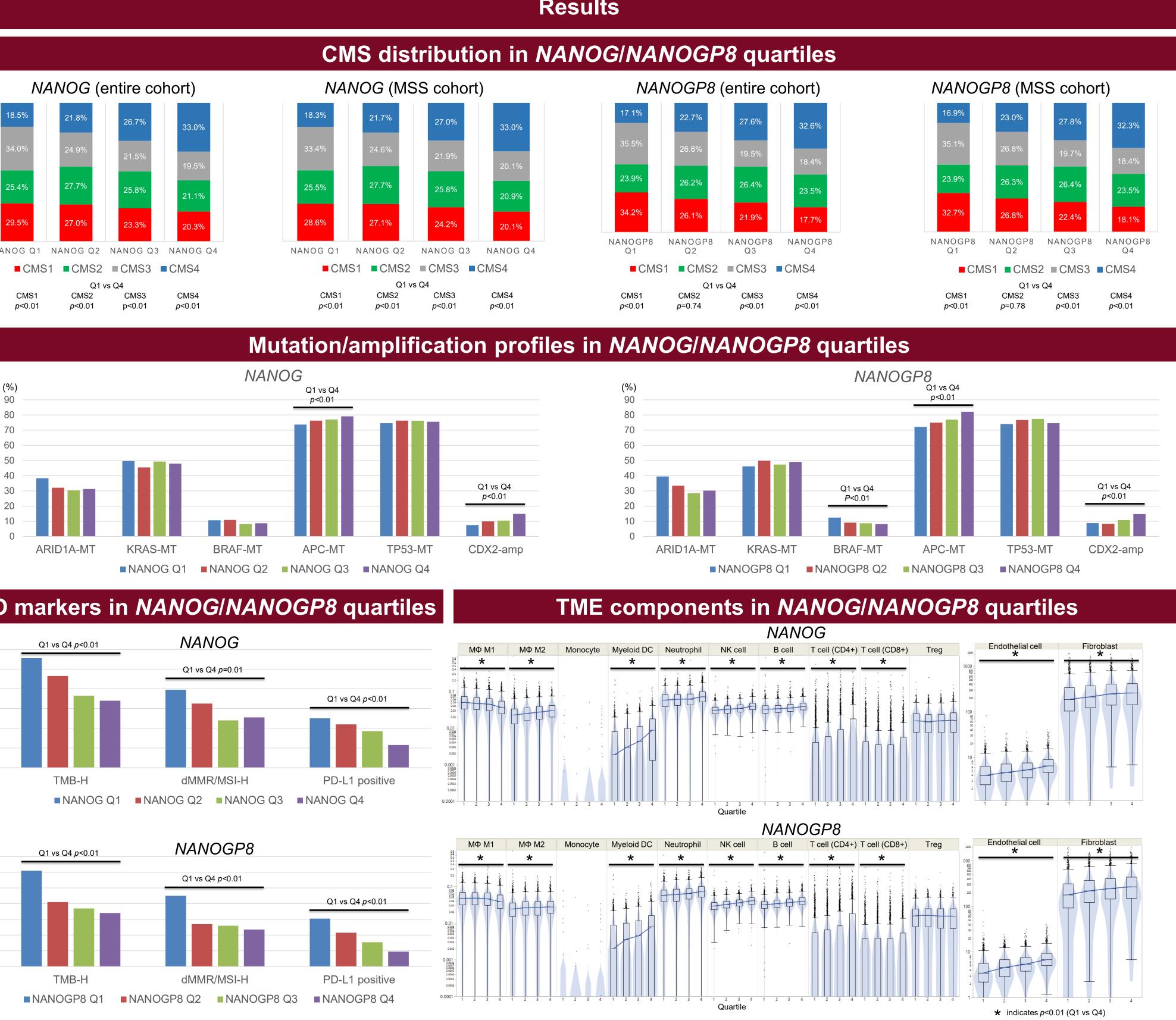
Introduction

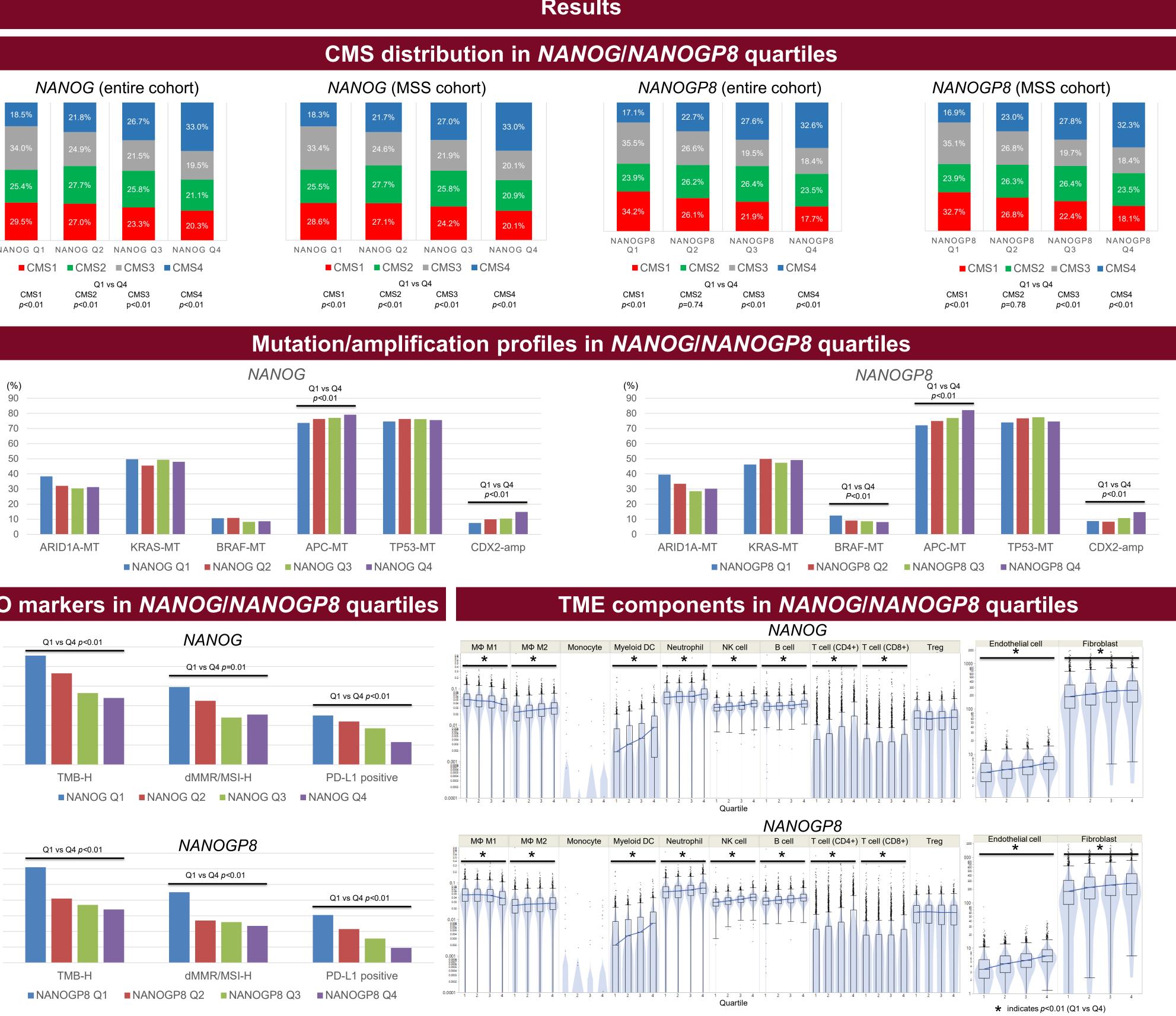
- The cancer stem cell (CSC) possesses self-renewal and multilineage differentiation potential, and believed to be responsible for resistance to chemotherapy and/or radiotherapy [1].
- NANOG is a pluripotency transcription factor that serves as a signaling hub in maintaining CSCs [2-3].
- Full-length NANOG protein is encoded by two paralogs of gene, namely NANOG1 (generally referred as NANOG) and NANOGP8 [4].
- NANOG mediates immune evasion through NANOG/TCL1A/AKT and NANOG/LC3B/EGFR axes, contributing to immune resistant phenotype [5-7].
- This study aimed to clarify molecular characters relating to gene expression levels of NANOG and NANOGP8 in patients with colorectal cancer (CRC).

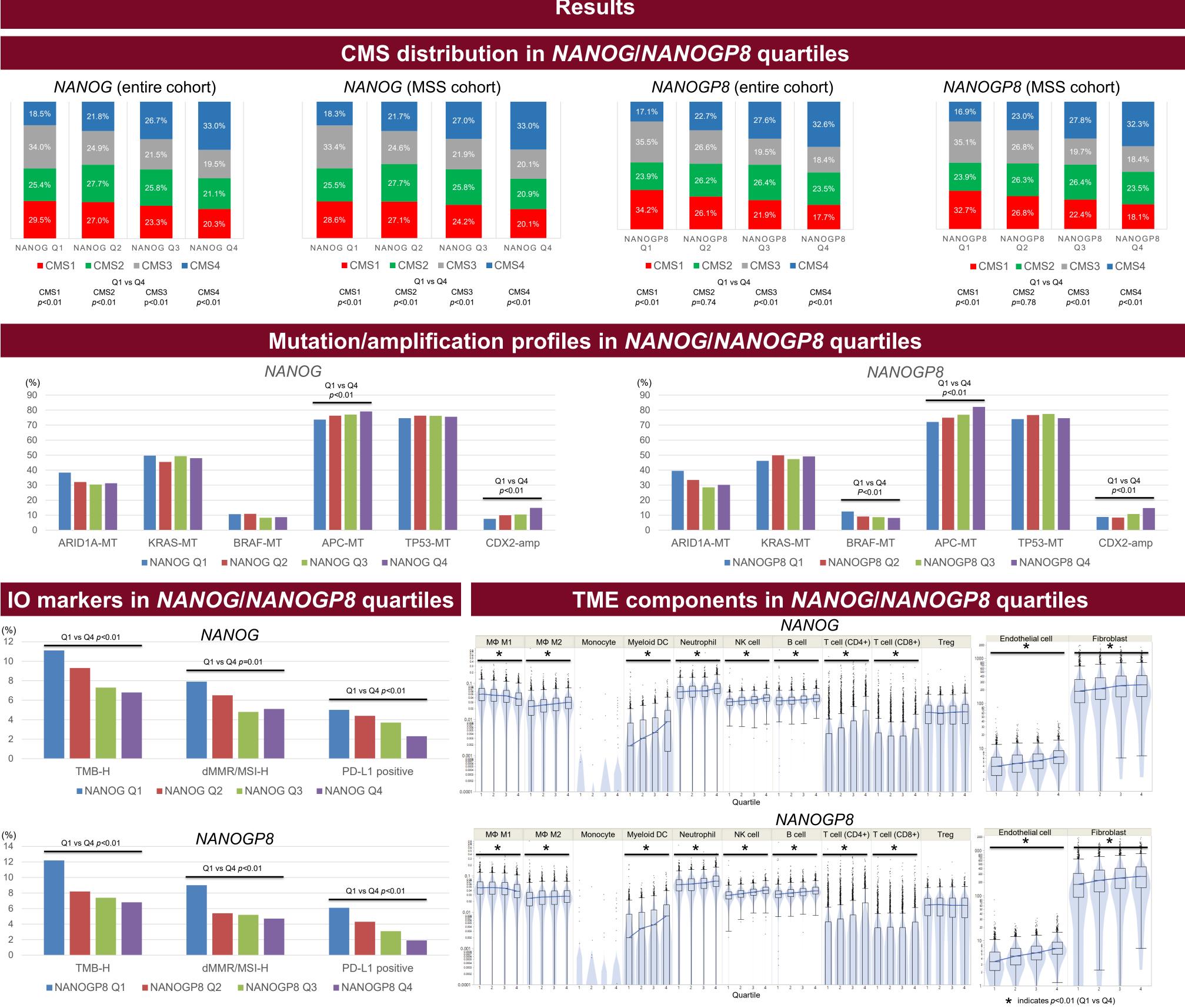
Methods

- Next-generation sequencing (NGS) and whole transcriptome sequencing (WTS) were performed on 7,604 CRC tumors submitted to Caris Life Sciences (Phoenix, AZ).
- Top quartile transcripts per million (TPM) for NANOG and NANOGP8 expression were considered high (Q4); while bottom quartile was classified as low (Q1).
- Consensus molecular subtypes (CMS) were identified using WTS data.
- Microsatellite instability (MSI) / mismatch repair (MMR) status was tested with a combination of NGS, immunohistochemistry (IHC) and fragment analysis.
- Tumor mutational burden (TMB) was measured by counting all nonsynonymous missense mutations found per tumor [592 genes and 1.4 megabases (MB) sequenced/tumor]. The threshold to define TMB-high (TMB-H) was ≥ 10 mutations/MB.
- PD-L1 was tested by IHC (using SP142 antibody) and tumor proportion score >5% was regarded as PD-L1 positive.
- Cell infiltration in the tumor microenvironment (TME) was assessed using QuantiSEQ and MCP counter.
- Molecular profiles were compared between Q4 and Q1. CMS distribution, mutation/amplification profiles, and immunotherapyrelated markers (IO markers: TMB, MSI/MMR status, and PD-L1 expression) were compared using Chi-Square or Fisher-Exact test. TME cell fractions were compared using non-parametric Kruskal-Wallis testing. Significance was determined by p < 0.05 after adjusting for multiple comparison.









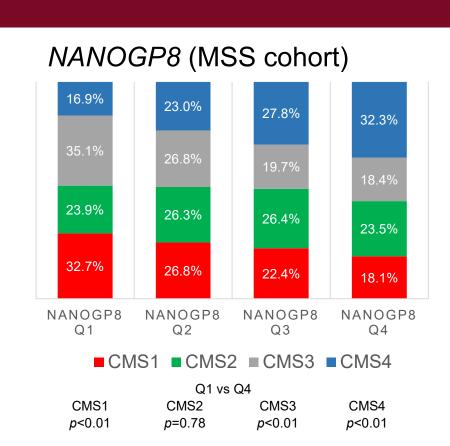


Gene expression of NANOG and NANOGP8 in colorectal cancer

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Summary



- CMS1, CMS2, and CMS3 were negatively associated with NANOG TPM (Q1 > Q4, p<0.01) while CMS4 had a positive association (Q4 > Q1: 33% vs. 19%, p<0.01). Similarly, CMS1 and CMS3 were negatively associated with NANOGP8 while CMS4 showed a positive association (33% vs. 17%). These associations were consistent in MSS cohort.
- APC mutations (NANOG Q4 vs. Q1: 79% vs. 74%; NANOGP8: 82% vs. 72%) and CDX2 amplifications (15% vs. 8%; 15% vs. 9%) were more frequently observed in Q4 than Q1 of NANOG and NANOGP8.
- Positivity rates of TMB-H (NANOG Q4 vs. Q1: 7% vs. 11%; NANOGP8 Q4 vs. Q1: 7% vs. 12%), dMMR/MSI-H (5% vs. 8%; 5% vs. 9%), and PD-L1 expression (2% vs. 5%; 2% vs 6%) were all negatively associated with both genes' TPM.
- In the TME, abundance of macrophage M1 was significantly lower in Q4 while that of myeloid dendritic cells, neutrophils, NK cells, B cells, T cells (both CD4+ and CD8+), endothelial cells, and fibroblasts was higher in Q4 compared to Q1 of NANOG and NANOGP8.

Conclusions

CRC harboring high expression levels of *NANOG* and **NANOGP8** genes was enriched in CMS4 and had a possible association with alterations in the WNT pathway. These tumors had an inflammatory TME which may lead to resistance to immunotherapy. Further investigations including clinical outcome data are warranted to reveal the clinical implications of NANOG.

References

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Disclosure statement / Funding

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