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Protocol for Retrospective Analysis of Circulating and Urine Tumor DNA Biomarkers in HCRN GU16-257: A Phase 2 Trial of Gemcitabine, Cisplatin, and Nivolumab with Selective Bladder Sparing in Muscle-Invasive Bladder Cancer

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Matthew D. Galsky, MD[1], Matthew Galsky[1]

[1]Icahn School of Medicine at Mount Sinai

Matthew Galsky
Icahn School of Medicine at Mount Sinai

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We use this protocol and it's working

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Abstract

HCRN GU16-257 was a prospective phase 2 trial evaluating gemcitabine, cisplatin, and nivolumab as organ-sparing treatment for muscle-invasive bladder cancer (MIBC). The trial enrolled 76 patients with cisplatin-eligible cT2-T4aN0M0 urothelial bladder cancer who received four cycles of treatment followed by clinical restaging. Patients achieving a clinical complete response (cCR) were offered the option to proceed without cystectomy and receive eight additional doses of nivolumab.

Circulating tumor DNA (ctDNA) and urine tumor DNA (utDNA) represent promising biomarkers for monitoring treatment response, predicting outcomes, and guiding therapeutic decisions in bladder cancer. This retrospective analysis aims to evaluate the prognostic and predictive value of these liquid biopsy biomarkers within the context of the organ-sparing treatment paradigm.

Guidelines

6.3.2 Survival Analyses
- **Method:** Kaplan-Meier survival estimates and log-rank tests
- **Effect Measure:** Hazard ratios with 95% confidence intervals from Cox proportional hazards models
- **Multivariable Analysis:** Cox regression adjusting for baseline prognostic factors
- **Landmark Analysis:** For restaging biomarker assessments, landmark analysis will be performed with landmark time set at the median time of restaging assessment

6.4 Secondary and Exploratory Analyses
6.4.1 ctDNA Kinetics Analysis
- Four-category analysis as defined above
- Pairwise comparisons between kinetics groups
- Trend analysis for ordered categories

6.4.2 Combined Biomarker Analysis
- Joint modeling of ctDNA and utDNA status
- Development of composite biomarker scores
- Classification tree analysis to identify optimal biomarker combinations

6.4.3 cCR Subgroup Analysis
- Kaplan-Meier survival estimates and log-rank tests

6.5 Descriptive Analyses
- Swimmer plots depicting individual patient trajectories
- Time-to-event curves stratified by biomarker status
- Waterfall plots showing biomarker changes from baseline to restaging

6.6 Handling of Missing Data
- Missing data patterns will be described

6.7 Multiple Comparisons
- All analyses will be interpreted at $\alpha = 0.05$ level

8.2 Data Verification
- Independent verification of key clinical endpoints
- Biomarker data cross-referenced with sample tracking logs
- Audit trail for all data transformations and derivations

8.3 Protocol Adherence
- This protocol represents a "locked-in" analysis plan
- Any deviations from the planned analyses will be documented and justified
- Post-hoc analyses will be clearly identified as such

8.1 Data Sources
- Parent trial clinical database (locked and validated)
- Biomarker laboratory databases (locked and validated)
- Long-term follow-up data collection

9.1 Primary Manuscript Structure
1. **Methods:** Detailed description of biomarker methodology and statistical approach
2. **Results:** Sequential presentation following primary → secondary → exploratory objectives
3. **Discussion:** Clinical implications and comparison with existing literature
4. **Limitations:** Acknowledgment of retrospective nature and potential biases

9.2 Supplementary Analyses
- Relationship between quantitative ctDNA and utDNA values (mutant allele fraction) and outcome
- Detailed baseline characteristics by biomarker status
- Complete survival curves and risk tables
- Additional descriptive statistics and visualizations

9.3 Clinical Interpretation Framework
- Clinical utility assessment for potential biomarker-guided treatment decisions

2.1 Primary Objectives

1 Determine the association between baseline ctDNA status (detectable vs. undetectable) and achievement of clinical complete response (cCR).

2 Determine the association between restaging ctDNA status and achievement of cCR.

3 Determine the association between restaging utDNA status and achievement of cCR.

2.2 Primary Objectives

4 Evaluate the association between baseline and restaging ctDNA status with metastasis-free survival (MFS) and overall survival (OS).

2.3 Primary Objectives

5 In patients achieving cCR, analyze the relationship between ctDNA kinetics and bladder-intact overall survival (BIOS)

2.3 Seconday Objectives

6 In patients achieving cCR, analyze the relationship between utDNA and BIOS

2.2 Secondary Objectives

7 Assess the relationship between ctDNA kinetics (persistent detectable, clearance from baseline to restaging) and MFS/OS.

2.3 Exploratory Objectives

7.1 In patients achieving a cCR, analyze the relationship between ctDNA kinetics and MFS/OS

2.2 Secondary Objectives

8 Evaluate the association between restaging utDNA status and MFS/OS.

2.3 Exploratory Objectives

9 Explore combined ctDNA and utDNA status as predictors of clinical outcomes.

10 Generate swimmer lane plots showing ctDNA/utDNA dynamics in relation to local and metastatic recurrence patterns in cCR patients.

3.1 Study Type

11 Retrospective cohort analysis of prospectively collected biospecimens and clinical data from HCRN GU16-257.

3.2 Study Population

12 All patients enrolled in HCRN GU16-257 with available ctDNA and/or utDNA data will be included in the analysis.

3.3 Biomarker Assessment Timeline

13 Baseline ctDNA: Collected prior to initiation of treatment (Cycle 1, Day 1).

14 Restaging ctDNA: Collected at time of clinical restaging (post-Cycle 4).

15 Restaging utDNA: Collected at time of clinical restaging (post-Cycle 4).

4.1 Assay Details

16 Platform: SaferSeqS

17 Assay Validation: The ctDNA and utDNA assays were "locked-in" prior to analysis.

18 Blinding: The biomarker analysis team was blinded to all clinical outcomes and response data.

19 Detection Threshold: Binary classification (detectable vs. undetectable) based on pre-specified assay cut-offs.

4.2 Quality Control Measures

20 Failed or inadequate samples will be documented with reasons for exclusion.

5.1 Response Endpoint

21 Clinical Complete Response (cCR): As defined in the parent trial:
- No evidence of malignancy on biopsy (except low-grade papillary Ta tumors)
- No malignant cells on urine cytology
- No evidence of local or metastatic disease on cross-sectional imaging

5.2 Survival Endpoints

22 Metastasis-Free Survival (MFS): Time from treatment initiation to first evidence of distant metastasis or death from any cause
Overall Survival (OS): Time from treatment initiation to death from any cause
Bladder-Intact Overall Survival (BIOS): Time from treatment initiation to death from any cause, with cystectomy treated as a competing risk (for cCR subgroup analysis)

5.3 ctDNA Kinetics Categories

23 Baseline Undetectable/Restaging Undetectable: Consistently undetectable

24 Baseline Detectable/Restaging Undetectable: Clearance

25 Baseline Undetectable/Restaging Detectable: Emergence

26 Baseline Detectable/Restaging Detectable: Persistent detectable

6.1 Sample Size and Power

27 This is a retrospective analysis utilizing all available samples from the parent trial. No formal sample size calculation was performed as this represents the complete available dataset.

6.2 Analysis Populations

28 Primary Analysis Population: All patients with evaluable ctDNA and/or utDNA data
Per-Protocol Population: Patients who completed all planned biomarker collections
cCR Subgroup: Patients who achieved clinical complete response (for exploratory analyses)

6.3.1 ctDNA/utDNA and Clinical Complete Response

29 Statistical Test: Fisher's exact test or Chi-square test
Effect Measure: Odds ratio with 95% confidence intervals
Multivariable Analysis: Logistic regression adjusting for baseline clinical factors (T-stage, age, ECOG performance status)

6.3.2 Survival Analyses

30 Method: Kaplan-Meier survival estimates and log-rank tests
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7.1 Baseline Clinical Factors

38 Age (continuous and categorized)
Sex

39 ECOG performance status
Clinical T-stage (cT2 vs. cT3-4)

7.2 Treatment-Related Factors

40 Achievement of clinical complete response

8.1 Data Sources

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9.3 Clinical Interpretation Framework

46 Clinical utility assessment for potential biomarker-guided treatment decisions

10.1 Regulatory Approval

47 This analysis was pre-planned as part of the parent trial's translational research objectives
Original informed consent included provisions for biomarker research

10.2 Data Privacy

48 Results will be reported in aggregate form only
Individual patient data will not be identifiable in any publications

11.1 Study Limitations

49 Retrospective analysis with inherent selection biases
Single-arm trial design limits comparative effectiveness assessment
Biomarker collection limited to pre-specified timepoints
Potential for informative missingness of biomarker data

11.2 Key Assumptions

50 Tumor-informed assays provide clinically relevant detection sensitivity
Binary classification (detectable/undetectable) captures clinically meaningful differences
Biomarker kinetics reflect underlying tumor biology and treatment response
Findings will be generalizable to similar patient populations and treatment regimens

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