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Methods

- Patients, male or female aged ≥ 18 years, treated with transurethral resection of bladder tumor (TURBT) and diagnosed with high-risk NMIBC (primary histologically confirmed T1 and/or high-grade tumors and/or carcinoma in situ) and be eligible for BCG therapy.

The diagram illustrates the study design for high-risk TNBC patients, divided into two parallel treatment arms. Both arms start at a baseline (BA) and follow a timeline with key events marked at Baseline (BA), M2, M4, M6, and FOLLOUP (48 weeks + 2 years).

- Top Arm (Orange):**
 - Placebo 1:** Administered at 10 days.
 - Placebo 2:** Administered at 6 days.
 - Intravenous DCO:** Administered weekly for 40 weeks.
- Bottom Arm (Blue):**
 - EMT1:** Administered at 10 days.
 - EMT1 2:** Administered at 6 days.
 - Intravenous DCO:** Administered weekly for 40 weeks.

The timeline for both arms includes Baseline (BA), M2, M4, M6, and FOLLOUP (48 weeks + 2 years).

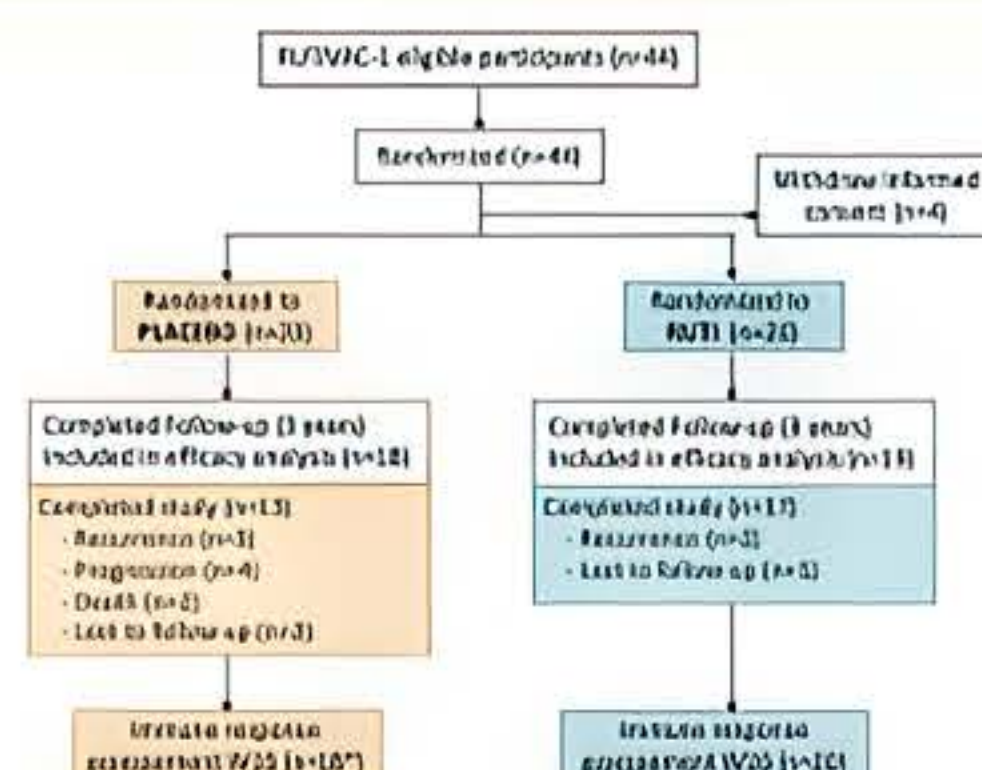
- ## Endpoints

- **Primary Endpoint:**
 - Evaluate the immunological response to RUTI vaccination prior to intravesical BCG therapy in patients with high-risk NMIBC.
- **Secondary Endpoints:**
 - Determine the efficacy of RUTI in reducing recurrence, progression, and death rates.
 - Evaluate the safety and tolerability of RUTI in patients with high-risk NMIBC.

Immune response assessment

- Activation-induced marker (AIM) analysis.
- *Ex vivo* expression of IFN- γ , IL-2 and TNF- α using intracellular cytokine staining (ICS).

Patient Disposition



Patient Demographic and Tumoral Characteristics

	Total population (n = 67)	Vaccine Administration		p-value
		HLI† (n = 23)	Pneumo (n = 20)	
Age (years), median (IQR)	70 (62-75)	71 (63-77)	67 (59-74)	0.25
Gender (male), n (%)	16 (24)	17 (85)	19 (95)	0.61
Weight (kg), median (IQR)	77 (67-83)	77 (70-84)	69 (60-81)	0.03
Body mass index (BMI) (kg/m²)	26 (23-28)	23.3 (21-26)	26 (23-28)	0.10
Smoking status, n (%)				
Nonsmoker (n, n/total)	7 (11)	6 (30)	1 (5)	0.09
Smoker	19 (28)	14 (70)	10 (50)	
Former smoker	22 (33)	10 (51)	12 (62)	0.72
Current smoker	11 (17)	4 (20)	7 (35)	
Other biomarkers: Pre-vaccination				
NLR, median (IQR)	2.1 (1.6-3.2)	2.1 (1.4-3)	2.1 (1.4-4)	0.74
PLR, median (IQR)	106 (98-150)	103 (78-151)	106 (68-210)	0.57
LNR, median (IQR)	2.9 (1.7-4.1)	2.4 (2.3-4.6)	2.5 (1.2-3.5)	0.12

	Total population (n = 65)	Vaccine status			p-value ^a
		10/10 ^b (n = 20)	19/20 ^c (n = 20)	1/20 ^d (n = 25)	
Cancer stage and grades, n (%)					0.16
T1N0	11 (22.5)	0 (10)	0 (20)	11 (22.5)	
T1G1	1 (2.5)	0 (0)	1 (5)	1 (2.5)	
T1N1	20 (40)	1 (10)	19 (20)	0 (0)	
Presence of concomitant CIL, n (%)					0.11
T1N1	4 (11)	0 (0)	4 (20)	0 (0)	
T1G1	1 (2.5)	0 (0)	1 (5)	0 (0)	
T1N0	5 (7.5)	0 (0)	2 (10)	3 (7.5)	
% of lesions, n (%)					0.045 ^{b,c}
Besley	21 (38.1)	1 (10)	1 (5)	19 (38.1)	
Mitotic	17 (45.3)	3 (25)	13 (65)	1 (2)	
No dysplasia	2 (5)	1 (5)	1 (5)	0 (0)	
Concomitant, n (%)					<0.001 ^{b,c}
None (0 mm)	10 (47.5)	10 (50)	0 (0)	0 (0)	
Large (≥1 mm)	11 (52.5)	2 (10)	0 (0)	9 (45)	
No dysplasia	9 (7.1)	5 (25)	1 (5)	3 (15)	
SCC% complete treatment, n (%)					1 (0.99) ^{b,c}
Yes	13 (62.1)	17 (83)	16 (80)	0 (0)	

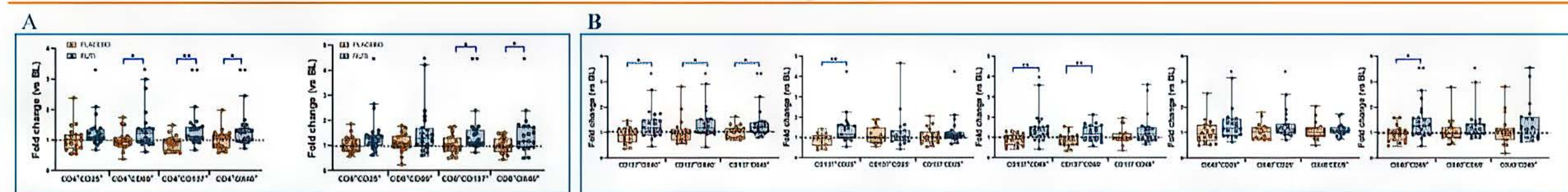


Figure 1. Modulation of the immune response by RUTI before BCG intravesical treatment. Fold change values (differences pre- and post-vaccination samples (BL and W2, respectively) in CD4⁺ and CD8⁺ T cells stimulated with PPD (A). Changes in the co-expression of AIMs⁺ in CD4⁺ T cells (B). Data are displayed as box and whiskers (5-95 percentile). Differences were tested using a Wilcoxon signed-rank test (differences compared to 1) and a Mann-Whitney U test for unpaired samples (differences between samples placebo and RUTI groups; blue lines). *p ≤ 0.05; **p ≤ 0.01

Boosted effect of RUTI Vaccine in the response to iBCG

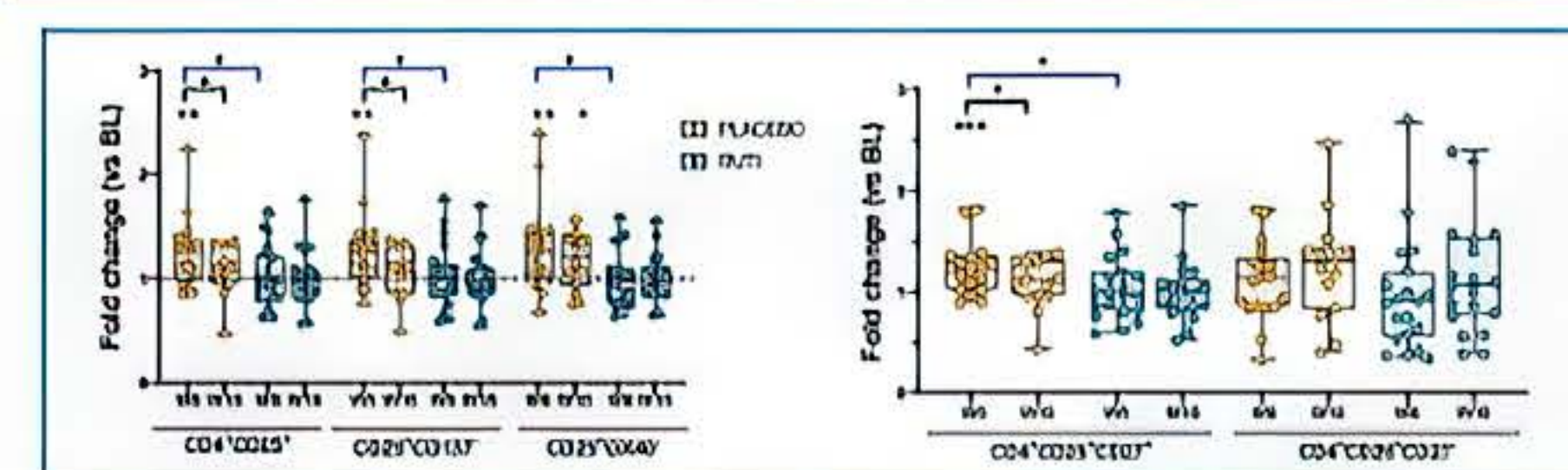


Figure 2. CD4⁺ and CD8⁺ T cell evolution without *ex vivo* stimulation. Fold change values (differences pre-inoculation (BL) and W6 and W16 in non-stimulated samples). Changes in the AIM profile of CD4⁺CD25⁺ and CD4⁺CD25⁺CD27⁺ T cells (Treg). Box and whiskers (5–95 percentile). Wilcoxon signed-rank test (differences compared to 1); Wilcoxon matched-pair signed-rank test (samples from the same individual; black lines). Mann-Whitney U test (differences between arms; blue lines).

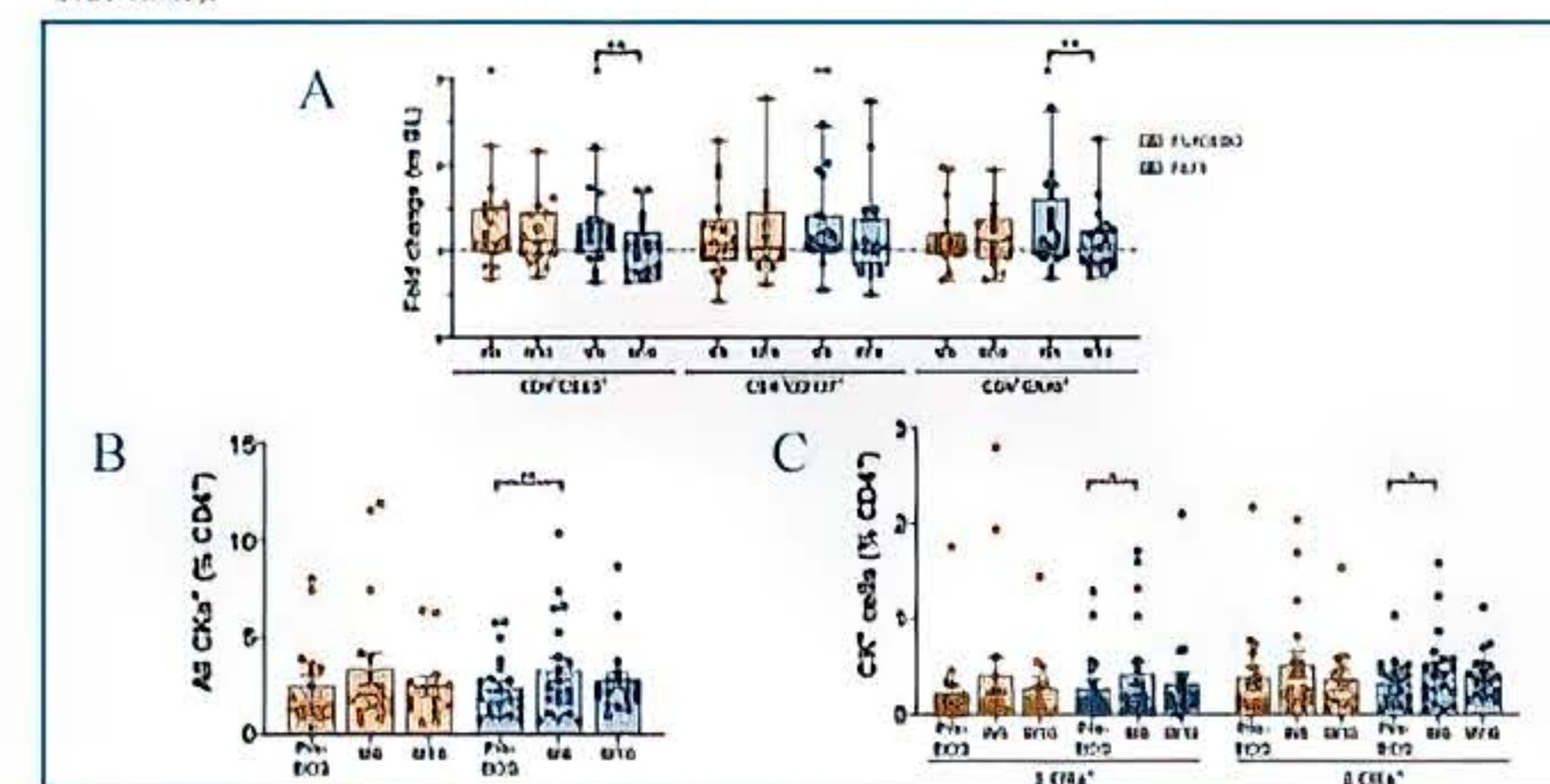


Figure 3. Antigen-specific CD4⁺ T cell response after ex vivo PPD stimulation. Fold change values in frequencies of AIM⁺ (CD25⁺, CD137⁺, and OX40⁺) at W6 and W16. Data as box and whiskers (5-95 percentile) (A). Frequencies of total cytokine (B) and cells producing multiple cytokines (triple and double-positive producing cells) (C). Data represent the mean (bars) and sEM (error bars) and each dot represent a patient. Wilcoxon test for comparisons between time points in the same individual. * $p < 0.05$; ** $p < 0.01$.

Treatment Outcome and Efficacy

	Vaccine Administered		p value
	RU11	PLACEBO	
THREE YEARS FOLLOW-UP			
Recurrent-free survival rate (RFS)	89.5	83.3	0.291
Progression-free survival rate (PFS)	100	77.7	0.032
Cancer-specific free survival rate (CSS)	100	88.9	0.141
Event-free survival rate (EFS)	89.5	72.2	0.185

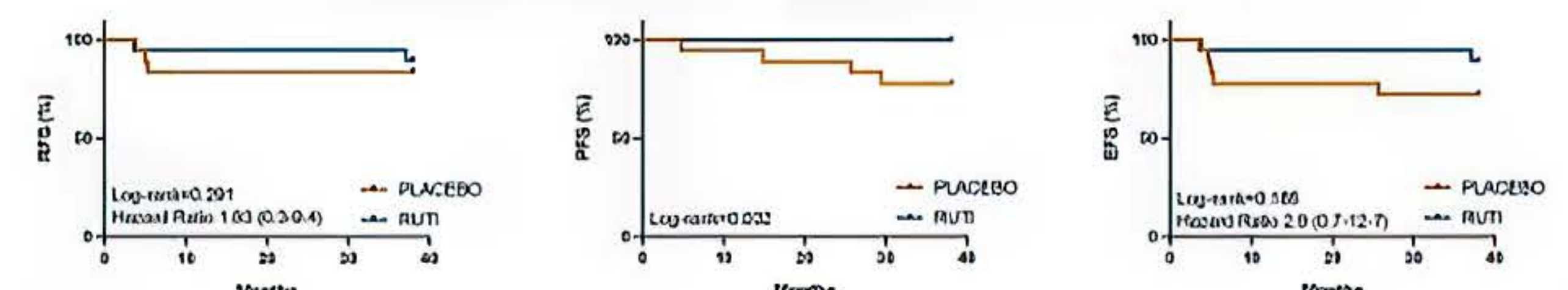


Figure 4. Kaplan-Meier curves at 3-year follow-up of placebo and RUTI vaccinated patients. Recurrence-free survival (RFS), progression-free survival (PFS) and event-free survival (EFS) for patients who received either placebo or RUTI vaccine after 3-year of follow-up are presented. Statistical significance was assessed using a log-rank test. Statistical analysis was performed using a log-rank test.

Safety

RUTI vaccine was safe and well tolerate Only mild adverse events (AEs) were observed in both arms.

Conclusions

RUTI® vaccination administered prior to intravesical BCG therapy in patients with high-risk NMIBC is safe, very well tolerated and has a significant impact on the immune response. The observed immunomodulation may be associated with the improved survival outcomes in RUTI-vaccinated patients.

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