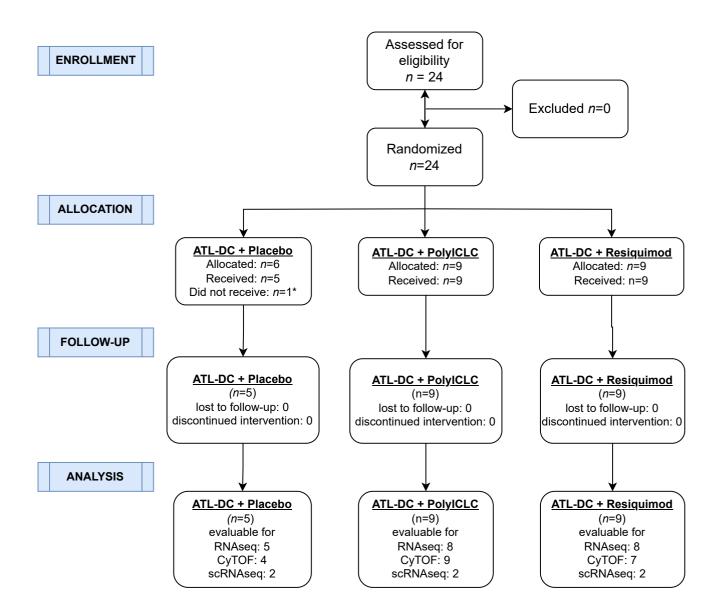
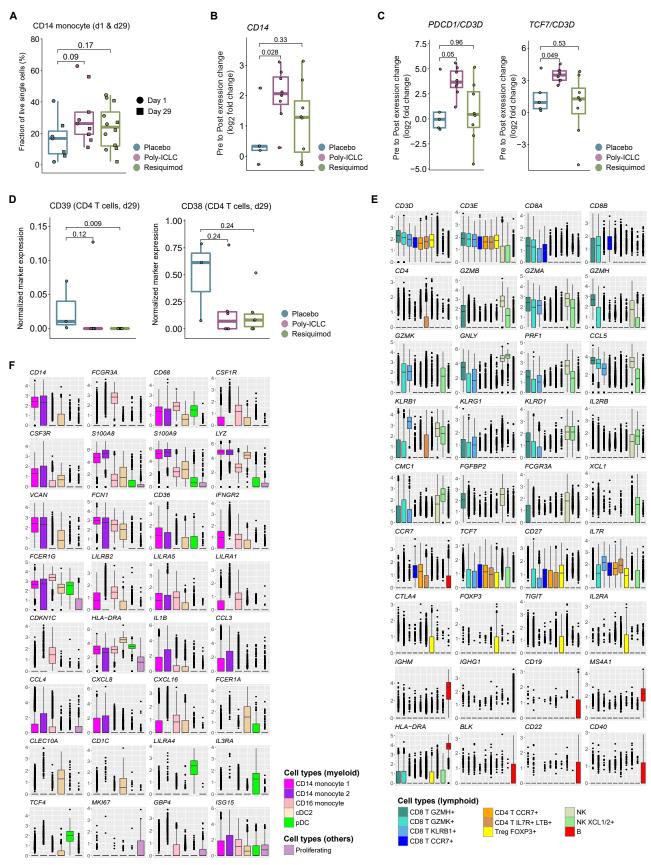
Supplementary Figure 1. CONSORT diagram of clinical trial enrollment.



\*due to early progression during vaccine manufacture

Supplementary Figure 1. CONSORT diagram of clinical trial enrollment.

## **Supplementary Figure 2**



**Supplementary Figure 2.** CyTOF and single cell transcriptomics of patient PBMCs before and after ATL-DC vaccine with or without adjuvant TLR agonist.

**A**, Comparison of CD14+ monocyte fraction in post-treatment PBMCs of patients from indicated treatment groups. P values, two-sided Wilcoxon rank sum test.

**B**, Differential gene expression (pre vs. post-treatment fold change, in log<sub>2</sub>) of *CD14* transcript across treatment groups (P values, two-sided Welch t test).

**C**, Differential gene expression (pre vs. post-treatment fold change, in  $log_2$ ) of *PDCD1* transcript across treatment groups (P values, two-sided Welch t test) after adjusting for the change in *CD3D* transcript expression in the same sample pair. The values approximate the changes of *PDCD1* transcript per T cell.

**D**, Normalized expression of indicated markers in CD4 T cell populations within the PBMC samples of patients from indicated treatment groups. P values, two-sided Wilcoxon rank sum test.

**E**, **F**, Boxplots showing marker gene expressions in lymphoid cell populations (**E**) or myeloid and proliferative cell populations (**F**).

The number of sample pairs analyzed by CyTOF in **A** and **D** are: ATL-DC+placebo, 4 pairs; ATL-DC+poly-ICLC, 9 pairs; ATL-DC+resiquimod, 7 pairs.

The number of sample pairs analyzed by RNAseq in panels B and C are: ATL-DC +placebo, 5 pairs; ATL-DC+poly-ICLC, 8 pairs; ATL-DC+resiquimod, 8 pairs.

The rectangular box in each boxplot represents the interquartile range (IQR), spanning from the first quartile (25<sup>th</sup> percentile, bottom of box) to the third quartile (75<sup>th</sup> percentile top of box). Inside the box, the median (50<sup>th</sup> percentile) is marked. The whiskers (shown as lines extending from the box) extend to the largest and smallest non-outlier values within 1.5 times the IQR, while outliers lie beyond the whiskers.

## **Supplementary Figure 3**

## Α

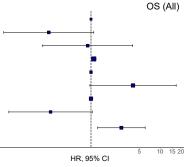
Tx Group	Placebo	_	
	Poly-ICLC	0.25 (0.05-1.10, p=0.066)	
	Resiquimod	0.89 (0.20-4.02, p=0.885)	
Age_at_DCVaxSurgery		1.10 (1.02-1.18, p=0.015)	
Grade	Ш	-	
	IV	4.06 (0.96-17.07, p=0.056)	
MGMT_methylation	FALSE	-	
	TRUE	0.26 (0.06-1.04, p=0.057)	-
RecurNum		2.75 (1.25-6.08, p=0.012)	
Tx_Group	Placebo	-	
	1 100000		

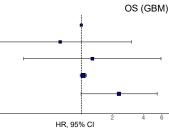
 Poly-ICLC
 0.63 (0.13-3.04, p=0.561)

 Resiquimod
 1.28 (0.28-5.89, p=0.751)

 Age\_at\_DCVaxSurgery
 1.04 (0.98-1.12, p=0.213)

 RecurNum
 2.32 (1.00-5.41, p=0.051)





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Pre-vaccination (Nov 2013)



Post-vaccination



Post-vaccination (Jun 2022, 9 years)

D



Pre-vaccination

(Apr 2014)





Post-vaccination (Jan 2023, 9 years)

**Supplementary Figure 3**. The association between combined ATL-DC vaccine and TLR agonist and patient survival.

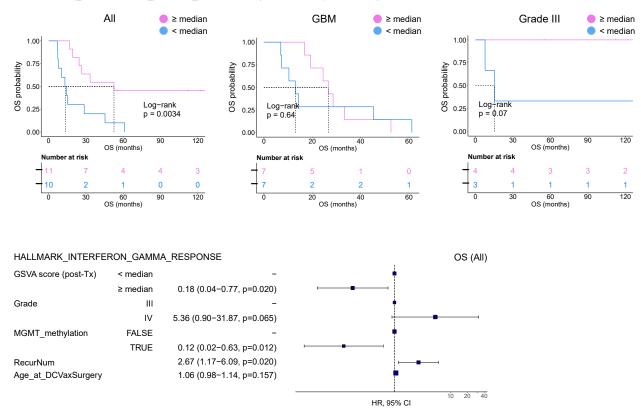
**A**, **B**, Multivariate Cox proportional hazards analysis assessing the hazard ratios of death in TLR agonist treatment groups against placebo in all patients (**A**) or GBM subset (**B**) after adjusting for other clinical covariates (Tx\_Group=treatment group, RecurNum=number of recurrences prior to ATL-DC treatment; the CoxPH model did not converge when MGMT\_methylation was included). In the forest plot, the squares are the hazard ratio (HR) estimates, the error bars are 95% confidence interval (CI) of the HR, the P value of each covariate is based on its Wald statistics, the P values are not adjusted. In (**A**), the sample distribution in each covariate is Tx\_Group: placebo=5, poly-ICLC=9, resiquimod=9; Grade: III=8, IV=15; MGMT\_methylation: True=8, False=15. In (**B**), Tx\_Group: placebo=4, poly-ICLC=5, resiquimod=6.

**C**, **D**, Representative contrast-enhanced MR imaging of patients treated with ATL-DC + poly-ICLC showing initial increase of T2/FLAIR MRI signal (red arrows), which either persists (**C**) or regresses (**D**) over time. Both patients have significantly longer PFS and OS than the rest of the patients in the cohort.

## **Supplementary Figure 4**

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A HALLMARK\_INTERFERON\_GAMMA\_RESPONSE (GSVA score, post-Tx, OS)



**Supplementary Figure 4**. The association between IFN pathway activation and overall survival after ATL-DC vaccine and TLR agonist therapy.

**A**, Kaplan-Meier overall survival curves of all patients (left), GBM (center), and Grade III glioma subsets (right) stratified by their

HALLMARK\_INTERFERON\_GAMMA\_RESPONSE GSVA scores in their posttreatment PBMCs. P values, log-rank test.

**B**, Multivariate Cox proportional hazards analysis assessing hazard ratios of death in patients with high HALLMARK\_INTERFERON\_GAMMA\_RESPONSE GSVA score after adjusting for other clinical covariates. In the forest plot, the squares are the hazard ratio (HR) estimates, the error bars are 95% confidence interval (CI) of the HR, the P value of each covariate is based on its Wald statistics, the P values are not adjusted. T the sample distribution in each covariate is GSVA score (post-Tx): <median=10, ≥median=11; Grade: III=7, IV=14; MGMT\_methylation: True=7, False=14.