Table SI. Key differences between the INSPIRE study and GU-specific retrospective analyses in chrono-immunotherapy.

Study	Design and population	Cancer types	Sample size	Timing definition	Outcomes reported	Effect estimates	Key findings	Key limitations and interpretations	(Ref.)
Investigator-initiated Phase II Study of Pembrolizumab Immunological Response Evaluation (INSPIRE)	Prospective phase II biomarker-rich pembrolizumab study. Followed by retrospective analysis of pembrolizumab-treated patients across multiple tumor types	Mixed (HNSCC, TNBC, ovarian, melanoma and others, including GU)	106	Multiple cut-offs, including noon, median ~3:00 p.m., ≥20% after 4:30 p.m. and seasonal cut-offs	OS and PFS	Not reported (no HR or CI values provided)	No association between infusion timing and outcomes	Limited sample size fragmented across tumor types (~12 patients per subgroup); heterogeneous biology; short follow-up (11.5 months); lack of HR and CI values limited interpretability; likely underpowered for tumor-specific effects	(76)
Molina-Cerrillo *et al*, 2022	Multi-center retrospective	mRCC	61	4:30 p.m.	PFS and OS	PFS demonstrated a HR value of 2.28 (95% CI, 1.1-5.15; P=0.048); the improved trend in OS exhibited a HR of 2.33	Late infusion associated with poorer PFS; OS showed a trend toward improvement with earlier administration	Limited sample size; immature OS; retrospective	(45)

Nelson *et al,* 2022	Large retrospective cohort	Advanced solid tumors (14.4% RCC)	4,441	Overnight versus daytime, which lasted from 10:00 a.m. to 4:00 p.m.	OS and PFS	Notable OS difference (renal subgroup P<0.05)	Overnight infusion associated with poorer OS in the RCC subgroup	Mixed tumor population; heterogeneous regimens (43% investigational); indirect GU signal	(46)
Ortego *et al*, 2022	Multi-center retrospective	mUC	92	4:30 p.m.	PFS, OS and ORR	PFS had a HR of 2.66 (95% CI, 1.53-4.63); OS exhibited a HR of 2.62 (95% CI, 1.48-4.63); P=0.001	Strong association favoring early infusion across all endpoints	Short follow-up of 8.6 months; retrospective	(47)
Fernández-Mañas *et al*, 2023	Single-center retrospective	mRCC	104	4:30 p.m.	OS and treatment duration	OS showed a HR of 3.1 (P=0.01)	Early infusion associated with markedly improved OS and disease control	Small late-infusion subgroup; retrospective	(48)
Dizman *et al*, 2023	Single-center retrospective	mRCC	135	≥20% after 4:30 p.m.	TTF, OS and ORR	TTF showed a HR of 1.694 (95% CI, 1.064-2.698; P=0.026)	Late infusion associated with poorer TTF; OS trended favorably towards earlier administration	Retrospective; OS was not statistically significant	(49)

Arroyave Ramirez *et al*, 2024	Single-center retrospective	mRCC	127	4:30 p.m.	OS and ORR	OS had a HR of 0.67 (95% CI, 0.51-0.83; P=0.03)	Early infusion associated with improved OS and ORR	Retrospective; single center	(50)
Patel *et al*, 2024	Multi-center retrospective	mRCC	201	12:00 p.m.	PFS and OS	≥20% infusions received before noon group demonstrated markedly improved PFS (HR, 0.70; P=0.04) and OS (HR, 0.57; P=0.043)	Robust association favoring early infusion; validated in MVA	Retrospective; heterogeneity in regimens	(51)
MOUSEION-09 meta-analysis	Meta-analysis of 4 studies	mRCC	545	Early versus late (study-defined)	OS	OS had a HR of 0.62 (95% CI, 0.50-0.72; P<0.001)	Consistent pooled benefit of early infusion	Based on retrospective studies; heterogeneity across studies	(103)

PFS, progression-free survival; OS, overall survival; GU, genito-urinary; HNSCC, head and neck squamous cell carcinoma; TNBC, triple-negative breast cancer; HR, hazard ratio; CI, confidence interval; mRCC, metastatic renal cell carcinoma; ORR, objective response rate; mUC, metastatic urothelial carcinoma; TTF, time to treatment failure; MVA, multivariate analysis.