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## CONTEXT

The immune system is under the tight control of the circadian timing system [1].

Retrospective studies suggest potential large benefit of proper timing of **immune checkpoint inhibitors (ICI)** against metastatic cancers[2].

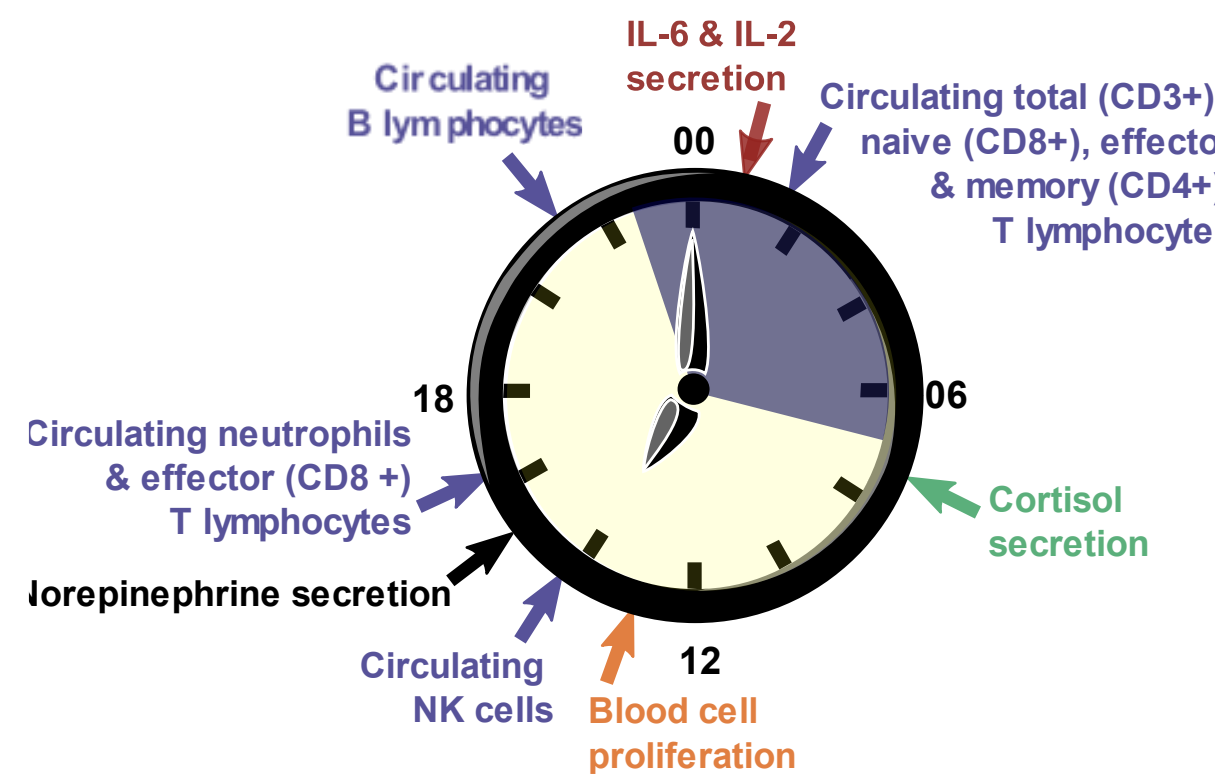


Fig. 1: Peaks over 24h of key elements of the immune system.

### AIMS:

- Retrospectively study association between ICI timing and patient overall survival (OS), neoplastic response and toxicities, and interactions with performance status (PS) and sex.
- Design new methodologies for analyzing timing-stipulated survival datasets

## Patient cohort and treatments

**Inclusion:** patients with metastatic or locally advanced solid tumors, who received pembrolizumab, nivolumab, atezolizumab, durvalumab, or avelumab, alone or with concomitant chemotherapy, (Nov 2015- Mar 2021, Centre Leon Bérard, France).

|                                               |                  | Missin | Overall          | Morning         | Afternoon        | P-Value |
|-----------------------------------------------|------------------|--------|------------------|-----------------|------------------|---------|
| Number of patients                            |                  |        | 361              | 136             | 225              |         |
| Median infusion time, median [Q1,Q3]          |                  | 0      | 12.3 [10.9,15.3] | 10.6 [9.8,11.1] | 14.9 [12.7,15.7] | <0.001  |
| Age (years), mean (SD)                        |                  | 0      | 62.5 (10.7)      | 61.7 (11.0)     | 62.9 (10.5)      | 0.324   |
| BMI, mean (SD)                                |                  | 2      | 23.8 (4.5)       | 24.2 (4.6)      | 23.6 (4.4)       | 0.210   |
| Sex, n (%)                                    |                  | 0      | 139 (38.5)       | 48 (35.3)       | 91 (40.4)        | 0.388   |
|                                               | Female           |        | 222 (61.5)       | 88 (64.7)       | 134 (59.6)       |         |
| Performance status, n (%)                     |                  | 1      | 298 (82.8)       | 124 (91.9)      | 174 (77.3)       | 0.001   |
|                                               | 0-1              |        | 62 (17.2)        | 11 (8.1)        | 51 (22.7)        |         |
| Immunoallergic history, n (%)                 |                  | 1      | 337 (93.6)       | 122 (90.4)      | 215 (95.6)       | 0.085   |
|                                               | No               |        | 23 (6.4)         | 13 (9.6)        | 10 (4.4)         |         |
| Histological type, n (%)                      |                  | 0      | 289 (80.1)       | 113 (83.1)      | 176 (78.2)       | 0.060   |
|                                               | NSCLC            |        | 3 (0.8)          | 1 (0.7)         | 2 (0.9)          |         |
|                                               | Colorectal       |        | 18 (5.0)         | 4 (2.9)         | 14 (6.2)         |         |
|                                               | Melanoma         |        | 22 (6.1)         | 3 (2.2)         | 19 (8.4)         |         |
|                                               | ENT              |        | 1 (0.3)          | 1 (0.7)         |                  |         |
|                                               | Breast           |        | 27 (7.5)         | 14 (10.3)       | 13 (5.8)         |         |
|                                               | Urinary          |        | 1 (0.3)          |                 | 1 (0.4)          |         |
| Pancreas                                      |                  | 168    | 68 (35.2)        | 27 (38.0)       | 41 (33.6)        | 0.612   |
| Tumor PDL1 expression, n (%)                  |                  |        | 51 (26.4)        | 20 (28.2)       | 31 (25.4)        |         |
|                                               | >1               |        | 74 (38.3)        | 24 (33.8)       | 50 (41.0)        |         |
|                                               | <1               |        | 126 (35.0)       | 51 (37.8)       | 75 (33.3)        | 0.458   |
| Previous systemic treatment, n (%)            |                  |        | 234 (65.0)       | 84 (62.2)       | 150 (66.7)       |         |
| Type of first immunotherapy, n (%)            |                  | 0      | 20 (5.5)         | 10 (7.4)        | 10 (4.4)         | 0.189   |
|                                               | Atezolizumab     |        | 22 (6.1)         | 11 (8.1)        | 11 (4.9)         |         |
|                                               | Durvalumab       |        | 132 (36.6)       | 41 (30.1)       | 91 (40.4)        |         |
|                                               | Nivolumab        |        | 186 (51.5)       | 74 (54.4)       | 112 (49.8)       |         |
|                                               | Pembrolizumab    |        | 1 (0.3)          |                 | 1 (0.4)          |         |
|                                               | Ipilimumab       |        | 294 (81.4)       | 107 (78.7)      | 187 (83.1)       | 0.363   |
| Concurrent chemotherapy, n (%)                |                  |        | 67 (18.6)        | 29 (21.3)       | 38 (16.9)        |         |
| Two sequential ICI, n (%)                     |                  | 0      | 351 (97.2)       | 132 (97.1)      | 219 (97.3)       | 1.000   |
|                                               | No               |        | 10 (2.8)         | 4 (2.9)         | 6 (2.7)          |         |
| Line of immunotherapy treatment, n (%)        |                  | 1      | 127 (35.3)       | 51 (37.8)       | 76 (33.8)        | 0.679   |
|                                               | 1st line         |        | 188 (52.2)       | 65 (48.1)       | 123 (54.7)       |         |
|                                               | 2nd line         |        | 33 (9.2)         | 14 (10.4)       | 19 (8.4)         |         |
|                                               | 3rd line         |        | 11 (3.1)         | 5 (3.7)         | 6 (2.7)          |         |
|                                               | 4th line         |        | 1 (0.3)          |                 | 1 (0.4)          |         |
|                                               | 5th line         |        | 344 (95.6)       | 125 (92.6)      | 219 (97.3)       | 0.064   |
| Treatment with anti-inflammatory drugs, n (%) |                  | 1      | 16 (4.4)         | 10 (7.4)        | 6 (2.7)          |         |
|                                               | No               |        | 2155 (43.2)      | 58 (43.3)       | 97 (43.1)        | 1.000   |
| Next treatment, n (%)                         |                  | 2      | 204 (56.8)       | 76 (56.7)       | 128 (56.9)       |         |
|                                               | No               |        | 168 (82.4)       | 61 (80.3)       | 107 (83.6)       | 0.691   |
| Type of next treatment, n (%)                 |                  | 157    | 1 (0.5)          | 1 (1.3)         |                  |         |
|                                               | Chemiotherapy    |        | 4 (2.0)          | 2 (2.6)         | 2 (1.6)          |         |
|                                               | Surgery          |        | 14 (6.9)         | 6 (7.9)         | 8 (6.2)          |         |
|                                               | Immunotherapy    |        | 17 (8.3)         | 6 (7.9)         | 11 (8.6)         |         |
|                                               | Radiotherapy     |        |                  |                 |                  |         |
|                                               | Targeted therapy |        |                  |                 |                  |         |

## Defining optimal morning/afternoon dichotomization according to Overall Survival

Two methods, based on individual patient median infusion times (i.e., >50% of ICI infusions before/after the cut-off):

- ❑ **Predictiveness curve method:** based on a univariable Cox risk model.
- ❑ **Maximize Restricted Mean Survival Times (RMST, i.e., areas under the survival curves up to ~90% of events had occurred) between groups, by grid search.**
  - Optimal morning/afternoon cut-off in terms of OS estimated at 11:37
  - Similar patient characteristics between two groups, apart from PS.

## Treatment duration, Response and Toxicities higher in morning group (vs afternoon) in PS0-1 patients

|                                     |                                               | Missing | Overall           | Morning            | Afternoon         | P-value all patients | P-Value PS0-1 | P-Value PS2-3 |
|-------------------------------------|-----------------------------------------------|---------|-------------------|--------------------|-------------------|----------------------|---------------|---------------|
| Response, n (%)                     | Progression                                   | 6       | 114 (32.1)        | 30 (22.7)          | 84 (37.7)         | 0.004                | 0.027         | 0.162         |
|                                     | Stability                                     |         | 74 (20.8)         | 26 (19.7)          | 48 (21.5)         |                      |               |               |
|                                     | Partial response                              |         | 127 (35.8)        | 61 (46.2)          | 66 (29.6)         |                      |               |               |
|                                     | Complete response                             |         | 40 (11.3)         | 15 (11.4)          | 25 (11.2)         |                      |               |               |
| Number of toxicities, n (%)         | None                                          | 0       | 218 (60.4)        | 69 (50.7)          | 149 (66.2)        | 0.006                | 0.024         | 0.202         |
|                                     | 1                                             |         | 98 (27.1)         | 48 (35.3)          | 50 (22.2)         |                      |               |               |
|                                     | 2                                             |         | 37 (10.2)         | 13 (9.6)           | 24 (10.7)         |                      |               |               |
|                                     | 3                                             |         | 8 (2.2)           | 6 (4.4)            | 2 (0.9)           |                      |               |               |
| Highest toxicity grade, n (%)       | Grade 0                                       | 0       | 218 (60.4)        | 69 (50.7)          | 149 (66.2)        | 0.008                | 0.028         | 0.203         |
|                                     | Grade 1                                       |         | 69 (19.1)         | 33 (24.3)          | 36 (16.0)         |                      |               |               |
|                                     | Grade 2                                       |         | 51 (14.1)         | 25 (18.4)          | 26 (11.6)         |                      |               |               |
|                                     | Grade 3                                       |         | 20 (5.5)          | 9 (6.6)            | 11 (4.9)          |                      |               |               |
|                                     | Grade 4                                       |         | 3 (0.8)           |                    | 3 (1.3)           |                      |               |               |
| Number of infusions, median [Q1,Q3] |                                               | 0       | 8.0 [4.0,20.0]    | 10.5 [5.0,23.0]    | 6.0 [3.0,19.0]    | 0.003                | 0.025         | 0.218         |
| Treatment duration, median [Q1,Q3]  |                                               | 73      | 98.0 [42.0,241.2] | 127.5 [49.0,328.8] | 75.5 [42.0,189.8] | 0.004                | 0.023         | 0.215         |
| Discontinue Reason, n (%)           | None (Ongoing)                                | 26      | 33 (9.9)          | 15 (12.1)          | 18 (8.5)          | 0.030                | 0.096         | 0.753         |
|                                     | Progression/Death/Palliative care             |         | 238 (71.0)        | 78 (62.9)          | 160 (75.8)        |                      |               |               |
|                                     | Switch maintenance/End upon patient's request |         | 31 (9.3)          | 16 (12.9)          | 15 (7.1)          |                      |               |               |
|                                     | Intolerance/Toxicity                          |         | 29 (8.7)          | 15 (12.1)          | 14 (6.6)          |                      |               |               |
|                                     | Other                                         |         | 4 (1.2)           |                    | 4 (1.9)           |                      |               |               |

## Superiority of morning ICI infusions in patients with PS0-1, but not with PS2-3

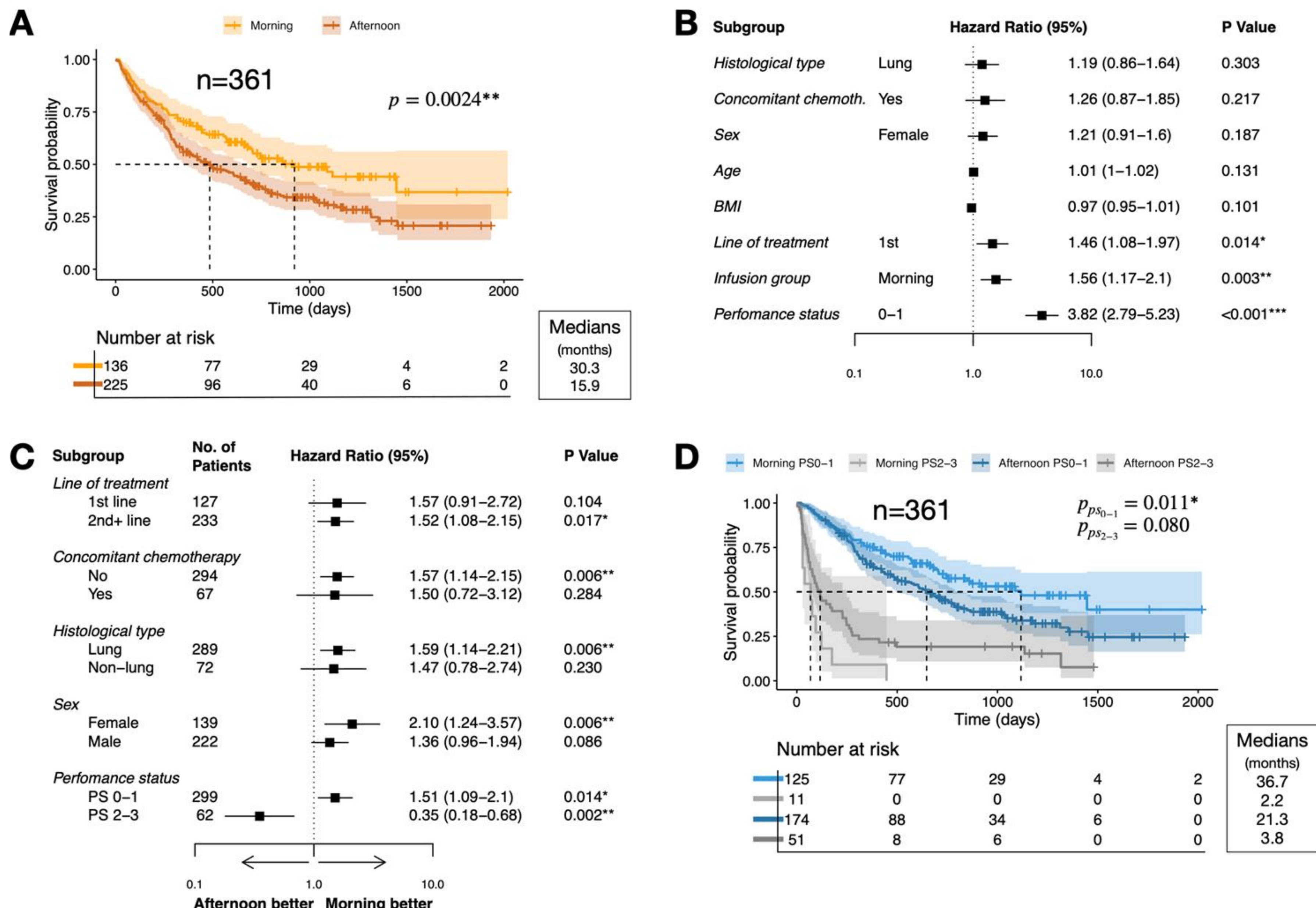


Fig. 2 : Association between ICI infusion timing and patient OS (A) OS of morning/afternoon groups, log-rank test (B) Forest plots of OS hazard ratios (HRs), univariable Cox models. (C) Forest plot of OS HRs by patient subgroups. HRs and 95% confidence intervals of an earlier death for afternoon vs morning infusion, univariable Cox models. (D) OS of morning/afternoon groups stratified by PS. Log-rank test for PS0-1 and Peto's log-rank test for PS2-3.

## Deciphering the Periodic Nature of ICI Timing Impact on Overall Survival of patients with PS0-1

- **Best (early morning) vs Worst (early afternoon) ICI timing resulted in a 4.8-fold difference in patient mortality risk.**

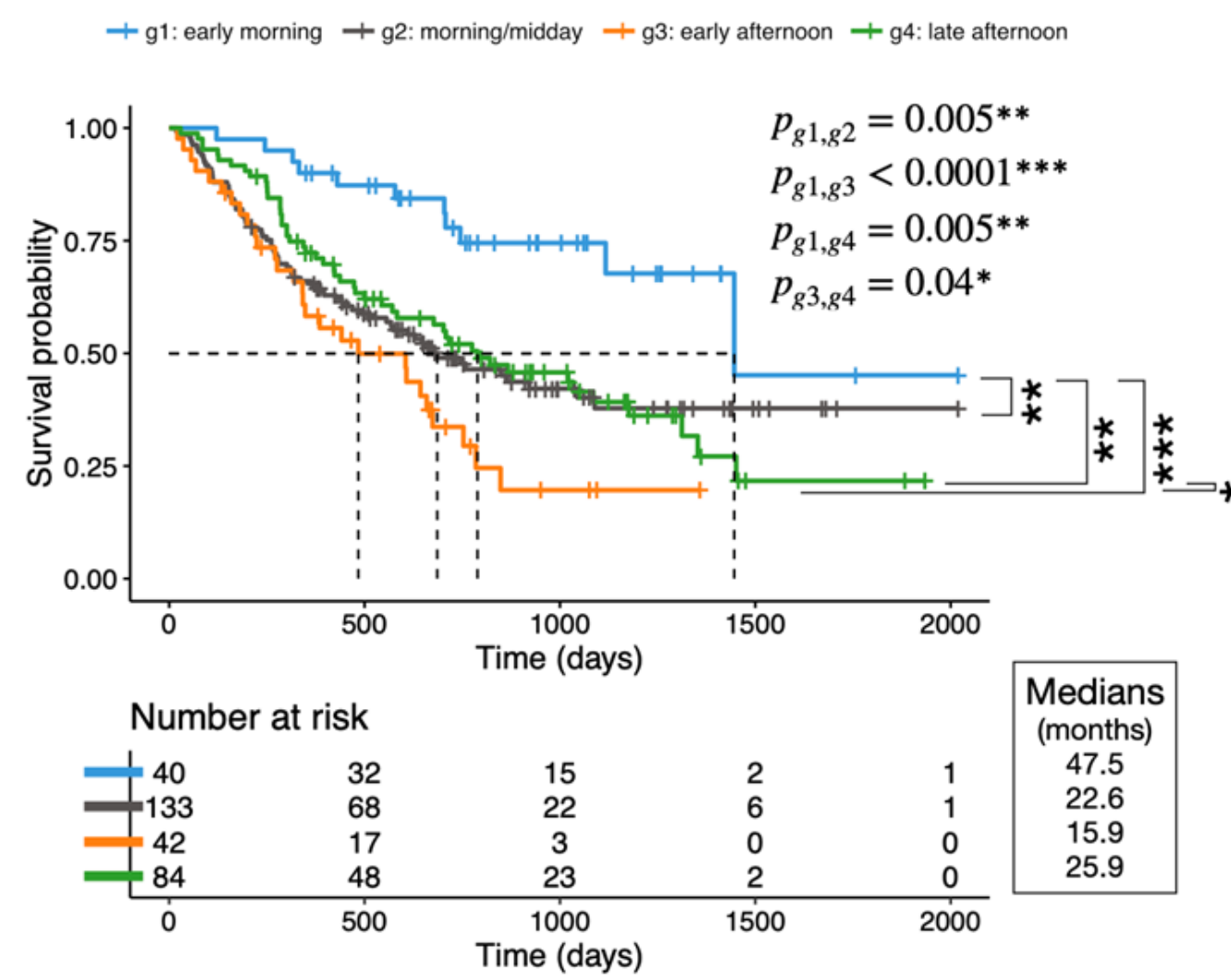


Fig. 3 : OS of patients with PS0-1 according to ICI timing. Kaplan-Meier curves for the four infusion groups defined as the ones maximizing pairwise differences in RMST. Optimal subgroups consisted in patients treated in the early morning (08:38-09:53, best survival, pairwise log rank p<0.005), morning/midday (9:53-12:40, intermediate), early afternoon (12:40-14:52, worst), and late afternoon (14:52-16:58, intermediate).

- **Periodic Cox regression to continuously model time-dependent mortality risk  $h(t)$  with respect to ICI timing  $\tau$ .**

$$h(t) = h_0(t)e^{A\cos(\frac{2\pi}{T}(\tau-\varphi))}$$

where  $T$  is the period, set to 24h,  $A$  is the amplitude (half-distance between minimum and maximum) and  $\varphi$  is the phase (time of peak). Parameter significance calculated by delta method.

- **Mortality risk ratio between infusions at worst time-of-day, estimated at 13:36 [12:48-14:23], and in early morning was predicted of 5.5-fold ([2-20], p=0.008).**

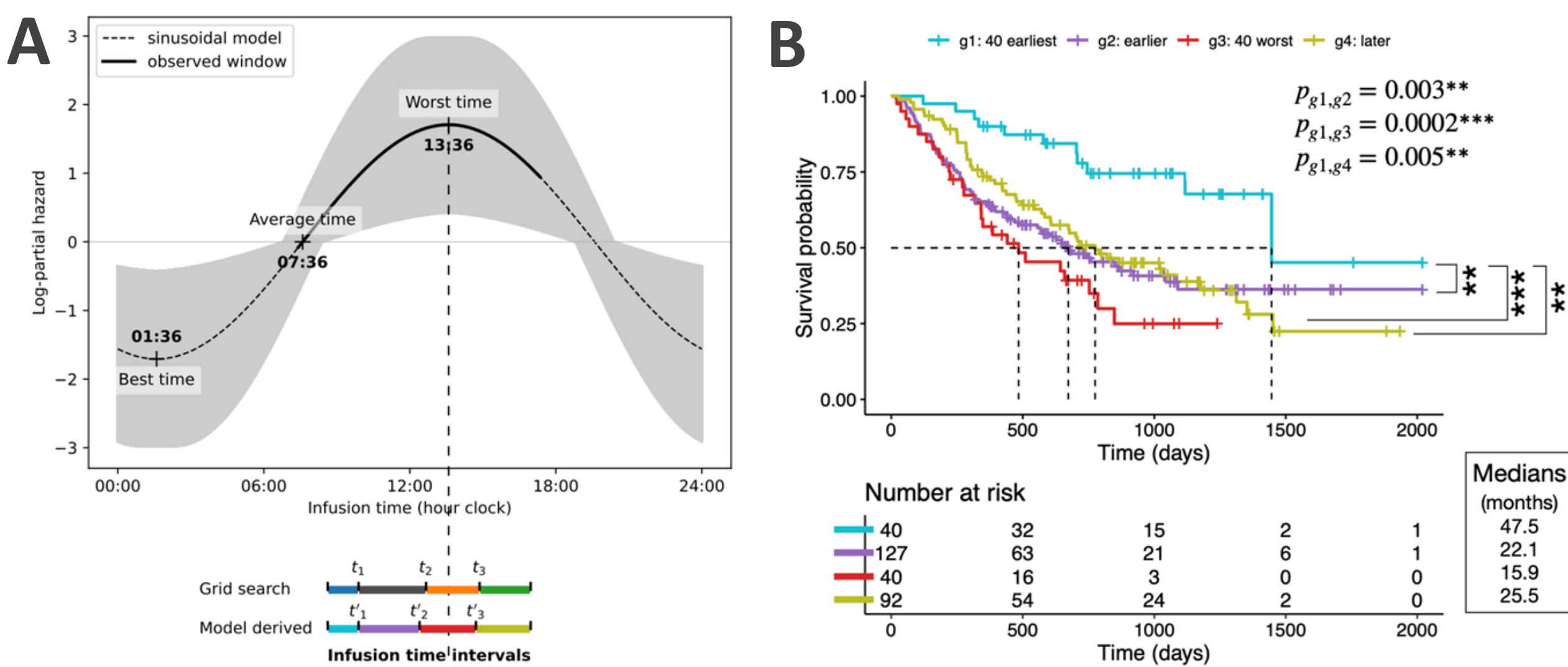


Fig. 4 : Periodic risk model to analyze OS of patients with PS0-1 (A) Log-partial hazard of the periodic Cox model. Shaded area is model 95% CI. (B) Kaplan-Meier curves for the four infusion groups defined using the periodic model. Timing cut-offs ( $t'_1$ = 09:53,  $t'_2$ =12:24,  $t'_3$ = 14:43) close to RMST-optimized ones (Fig. 3).

## CONCLUSION & PERSPECTIVES

- **Early morning ICI infusion was associated with increased OS, response, and toxicities in patients with PS0-1 as compared to later infusions within the day.**
- **Prospective randomized trials are needed to confirm this retrospective study.**
- **Ongoing work: design of ICI Quantitative Systems Pharmacology models accounting for circadian rhythms to inform on molecular mechanisms explaining timing-dependencies of patient outcomes.**

### References:

- [1] Circadian Regulation of Drug Responses: Toward Sex-Specific and Personalized Chronotherapy. FA Lévi, et al. Ann Rev Pharmacol Toxicol 2024 Jan. DOI: 10.1146/annurev-pharmtox-051920-095416
- [2] Early morning immune checkpoint blockade and overall survival of patients with metastatic cancer: An In-depth chronotherapeutic study. S Catozzi et al. Eur J Cancer 2024 Jan. DOI: 10.1016/j.ejca.2024.113571

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