



Prognostic Biomarker Signatures of Relapse in NMOSD

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Executive Summary

Longitudinal serum samples from 126 CIRCLES patients diagnosed with NMO were analyzed through mass spectrometry to identify circulating proteins associated with NMO relapse events.

This presentation summarizes the methods and results from the analysis of the proteomic data acquired from these samples.

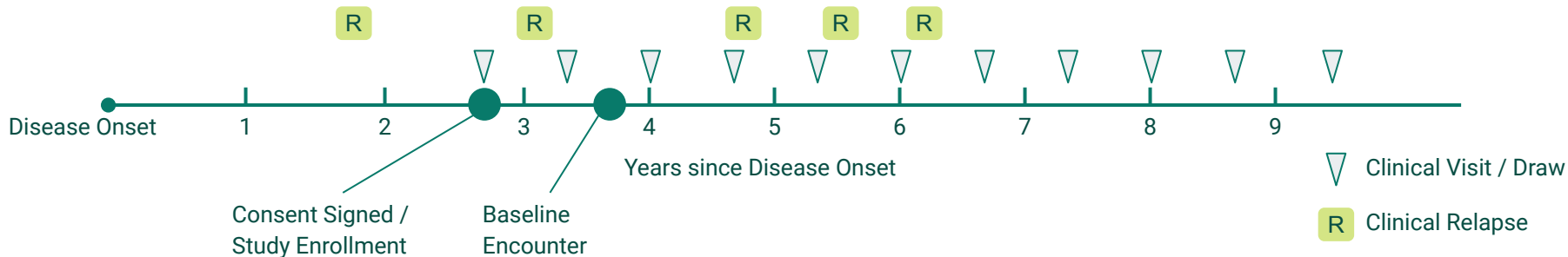


Agenda

- Experimental Design
- Cohort Characterization
- Modeling Techniques
- Results
- Conclusions

Longitudinal Samples Selected from CIRCLES Study

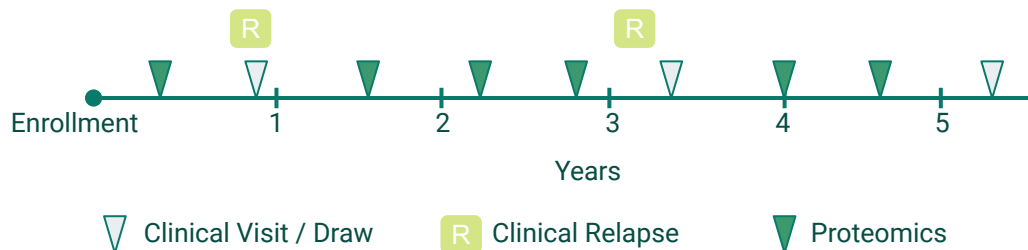
- Samples for this study were a subset of samples collected from NMO patients enrolled in the CIRCLES study. Selected NMO patients were diagnosed 2-10 years before joining CIRCLES.
- After consenting to enroll in the study (enrollment date), patients undergo a baseline encounter, after which they are officially part of the study.
- For each patient, biospecimens were collected at 6-month intervals.
- Relapses were evaluated regardless of these intervals and validated by neurologists.



Samples Were Excluded Based on Proximity to Relapse

Exclusion criteria:

- Samples collected at the time of relapse.
- Samples collected within 180 days of previous relapse.
- Last samples collected without a subsequent relapse.
- Samples without treatment information.

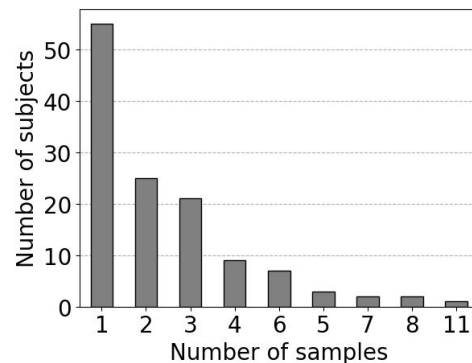


Samples Were Processed in 4 Experimental Batches

- In total, **305 samples** were processed through the proteomic pipeline in 4 experimental batches:
 - A uniform distribution of demographics and time-to-relapse events across batches was ensured to mitigate batch effects.

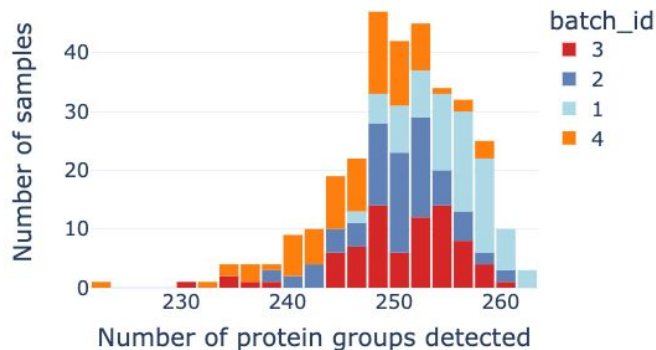
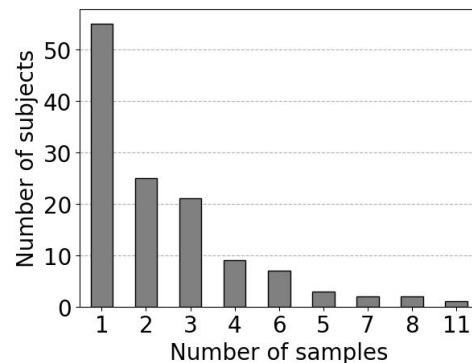
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- For half the patients only one sample was available for proteomics.
- 1 sample excluded due to quality issues.
- 265 proteins detected across all samples.

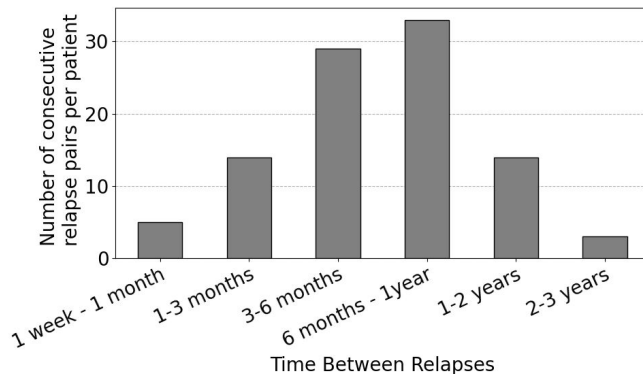


Study Demographics Aligned with NMO Demographics

- Majority of the patients are females (86%);
Average age at onset was 35 years;
64% anti-AQP4+, 8% anti-MOG+.
- Race breakdown:
 - White (52%), Black or African American (25%), Hispanic or Latino (14%), Asian (7%).

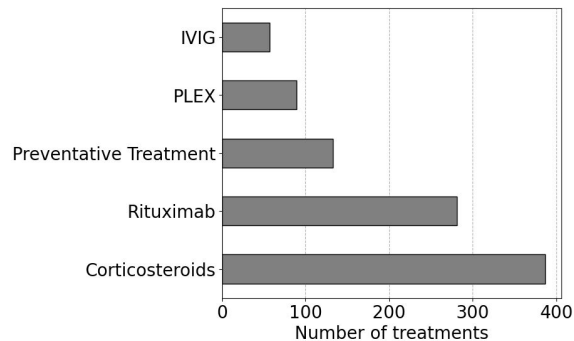
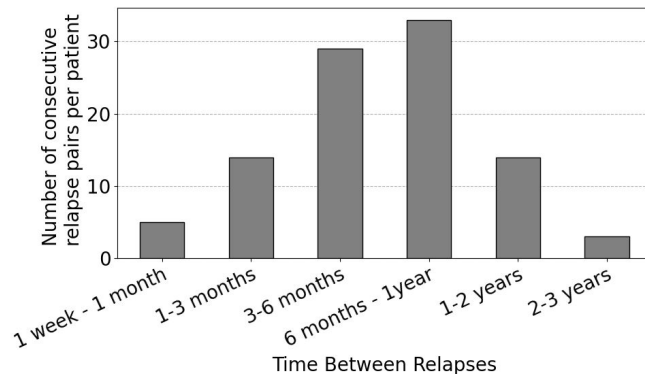
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- Since enrollment we observed 225 relapse events across all patients (avg 1.8 per patient).
- Relapses were usually re-occurring between 3 months and a year.



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- Relapses were usually re-occurring between 3 months and a year.
- Patients received acute and long-term treatments, which were divided in 5 groups for analysis.



Multiple Models to Identify Consistent Biomarkers

Patient timelines contain multiple types of information to include in computational models.

Data available for each patient:

- **Events:**

- Time-varying {
- Relapses
 - Sample collection, ie proteomics & blood contamination indices
 - Treatments (start and end dates)

- **Covariates:**

- Patient level constants {
- Sex
 - Race
 - Antibody status (APQ4 & MOG)
 - Proteomics batch

Modeling techniques with increasing levels of complexity:

1. Binary classification
2. Survival Analysis with Naive Cox Models
3. Time-Varying (time-dependent) Survival Analysis

Modeling 1: Logistic Regression

Assign a label to each sample: *will the patient experience a relapse in the next ... days after sample collection?* and fit a logistic regression model.

Threshold = 120 days (~4 months) → This yields ~20% of positive labels.

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Pros

- Easy interpretation of the model.
- Easy access to a probability of relapse.
- Simple dataset - one row per sample.

Cons

- Does not take into account treatment information.
- Relies on selection of threshold.
- With the current threshold, unbalanced classes.

Modeling 2: Survival Analysis with Naive Cox Models

The Cox model can be seen as an extension of traditional regression models that take into account censoring, ie the patient did not experience the event before the end of the study.

Each sample is **independently** assigned to its next relapse or censoring event. We fit a simple Cox Model Proportional Hazards model to this survival data.

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Pros

- Easy interpretation of the model.
- Takes into account censoring events.
- Simple dataset - one row per sample.

Cons

- Does not take into account treatment information.
- Each interval is considered independently even if they belong to the same patient timeline.

Modeling 3: Time-Varying Survival Analysis

Similar to the Naive Cox Model analysis, but **models the entire patient timeline**:

- The timeline is divided into non-overlapping intervals to fit a time Time-Dependent Cox model.
- **Treatments** are included in interval design.

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Similar to the Naive Cox Model analysis, but models the entire patient timeline:

- The timeline is divided into non-overlapping intervals to fit a time Time-Dependent Cox model.
- Treatments are included in interval design.

Pros

- Takes into account treatment data.
- Takes into account precise interval durations and censoring events.
- Takes into account full patient timeline.
- Does not consider samples independently.

Cons

- Prediction in a time-varying setting is not trivial.
- Complex dataset and intensive pre-processing.

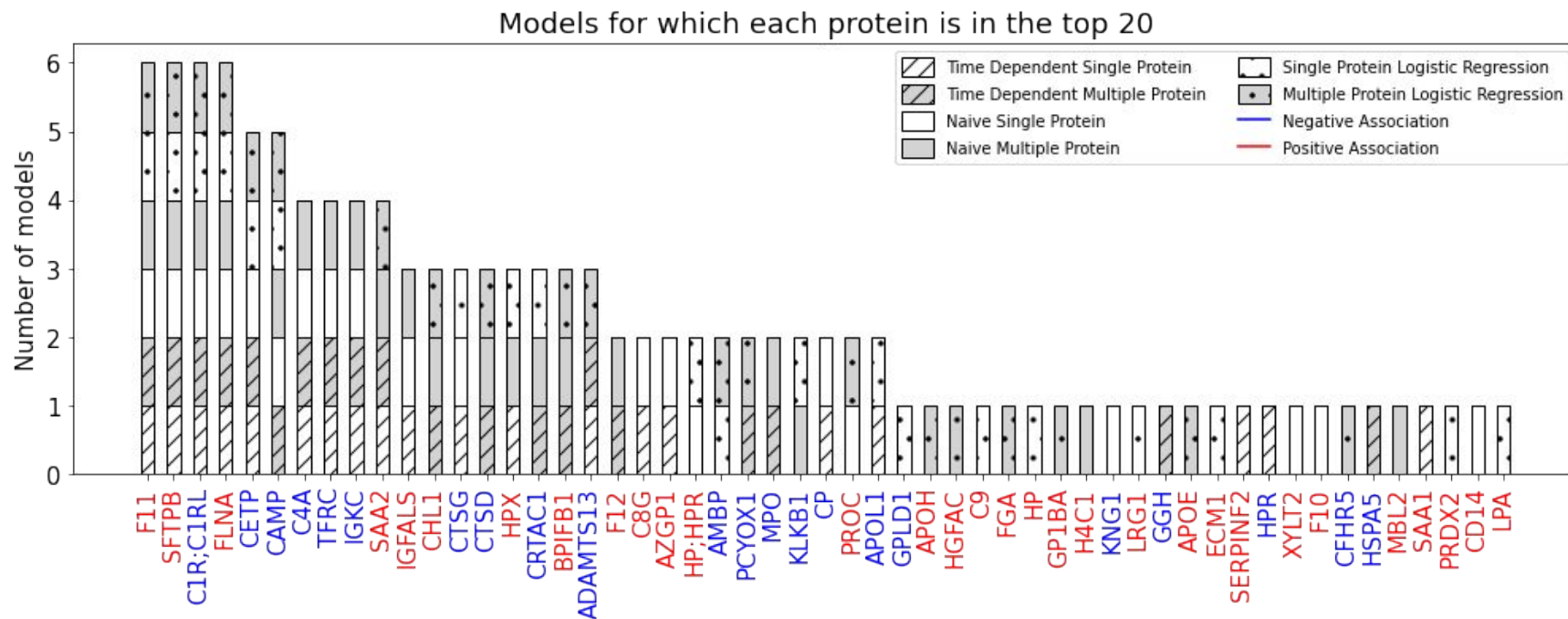
Additional Modeling Considerations

- Both multiple and single protein modeling were used:
 - **Multiple Protein:** Fit one model on the full data with all the proteins. In this case L1 regularization was used to help with feature selection and dimensionality reduction.
 - **Single Protein:** Fit 265 independent models, taking into account only the expression from one given protein at a time.
 - Previously defined covariates, such as demographics information, were included in both models.

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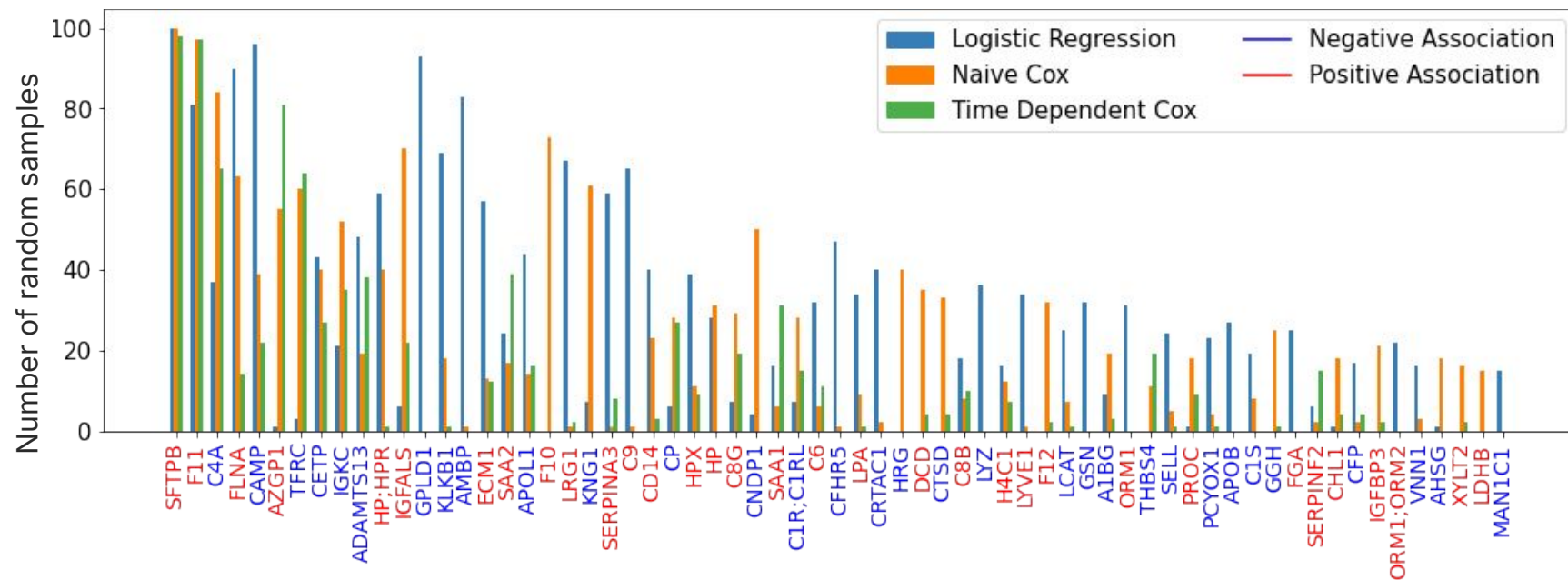
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 - **Single Protein:** Fit 265 independent models, taking into account only the expression from one given protein at a time.
 - Previously defined covariates, such as demographics information, were included in both models.
- **Random sampling** with replacement: we randomly sampled **80% of the subjects** and ranked the genes based on a given random subset for all models. We repeated this 100 times.
 - Ranking techniques: Genes were ranked by absolute average coefficient across repetitions for each model.

Proteins with Strongest Association with Relapse Are Consistent Across Modeling Strategies



Single-Protein Models Yields Consistent Biomarkers

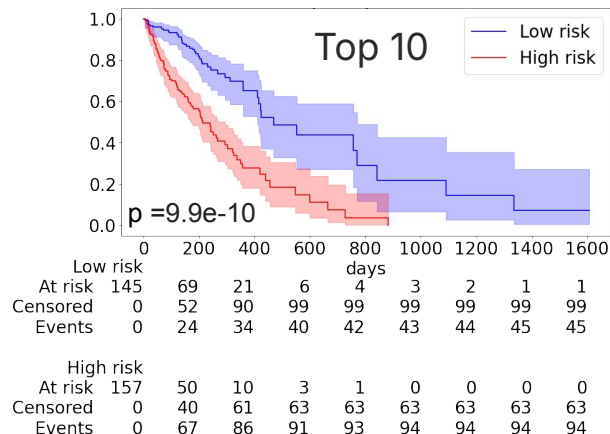
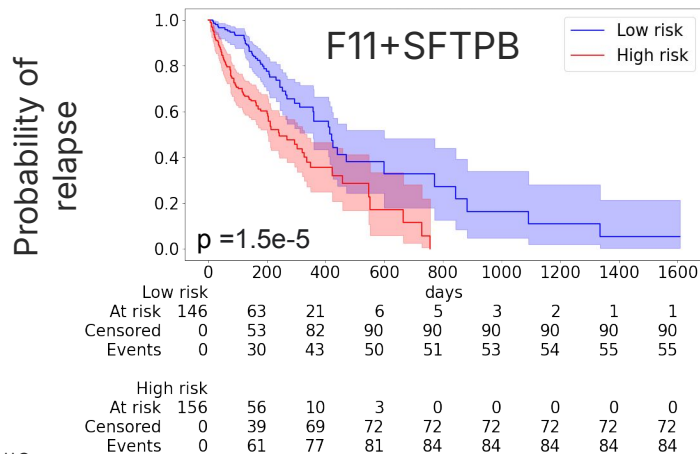
Proteins are ranked by the number of random samples where they had non-adjusted p-value below 0.05 (not possible with L1-regularized multiple-protein models).



Proof-of-Concept Risk Score Based on Top Proteins

Proteins with the strongest association with relapse might be used to build a relapse risk score. The median predicted survival time from this cohort can be used as a cutoff for stratifying patients into high and low risk groups.

This **will require additional validation** experiments to quantify selected biomarkers in a low throughput quantitative setting, further analysis to optimize the protein signature and an external validation cohort to test the predictive power of such a risk score.



Many Candidate Proteins Were Reported in Previous NMO Studies

- **Coagulation and complement pathways:** The interplay between coagulation and complement pathways is an emerging line of research [\[1\]](#):
 - Factors XI (**F11**), X (**F10**) XII (**F12**) and filamin A (**FLNA**) are involved in coagulation and have been shown to be differentially expressed in multiple sclerosis and NMO patients ([\[2\]](#), [\[3\]](#), [\[4\]](#)). They are positively associated with NMO relapses in our analysis
 - Complement 1 (**C1R**) and complement 4 (**C4A**) are complement proteins that have already been identified as biomarkers of interest in NMO [\[5\]](#). They are negatively associated with relapses in our analysis

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- **Cerebrospinal fluid (CSF) and nervous system damage:** These markers might pass the blood brain barrier which is more permeable in NMO patients [\[6\]](#):
 - Surfactant protein B (**SFTPB**) is predominantly produced in the lungs, but has also been detected in the central nervous system and CSF [\[7\]](#).
 - Serum amyloid A (**SAA1/SAA2**) is increased in response to neuroinflammation [\[8\]](#) and has been reported in relationship to NMO and multiple sclerosis in other studies [\[9\]](#).

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More literature search and experiments are needed to substantiate a biological explanation for the association of the top candidate proteins and NMO relapse.

Conclusions

- Thanks to this collaboration, we generated the largest collection of proteomics data from serum samples of NMO patients: ~300 samples from 100+ patients.
- Multiple modeling techniques and sampling were used to obtain a more robust set of potential biomarkers.
- Two proteins, F11 and SFTPb, emerged at top candidate biomarkers associated with NMO relapse.
- Several top candidate proteins are involved in coagulation pathways and neural damage response.
- **Further experiments and analysis** will be needed to confirm the top candidates as biomarkers of NMO relapse.

Acknowledgements



Michael R. Yeaman

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The Patients



Elea Bach

Yuliang Wang

Megan Carroll

Charles C. Kim

References



References

1. [Coagulation and complement: Key innate defense participants in a seamless web](#)
2. [Thrombin generation and activity in multiple sclerosis](#)
3. [Identification of Novel Serum Proteins Associated with Myelination and Cholesterol Transport in Neuromyelitis Optica Spectrum Disorders by Mass Spectrometry](#)
4. [Quantitative proteomic analyses of CD4 + and CD8 + T cells reveal differentially expressed proteins in multiple sclerosis patients and healthy controls](#)
5. [Plasma Complement 3 and Complement 4 Are Promising Biomarkers for Distinguishing NMOSD From MOGAD and Are Associated With the Blood-Brain-Barrier Disruption in NMOSD](#)
6. [Blood-brain barrier resealing in neuromyelitis optica occurs independently of astrocyte regeneration - PMC](#)
7. [The Detection of Surfactant Proteins A, B, C and D in the Human Brain and Their Regulation in Cerebral Infarction, Autoimmune Conditions and Infections of the CNS | PLOS ONE](#)
8. [Serum Amyloid A Protein as a Potential Biomarker for Severity and Acute Outcome in Traumatic Brain Injury - PMC](#)
9. [Serum amyloid A level is increased in neuromyelitis optica and atypical multiple sclerosis with smaller T2 lesion volume in brain MRI](#)

Appendix

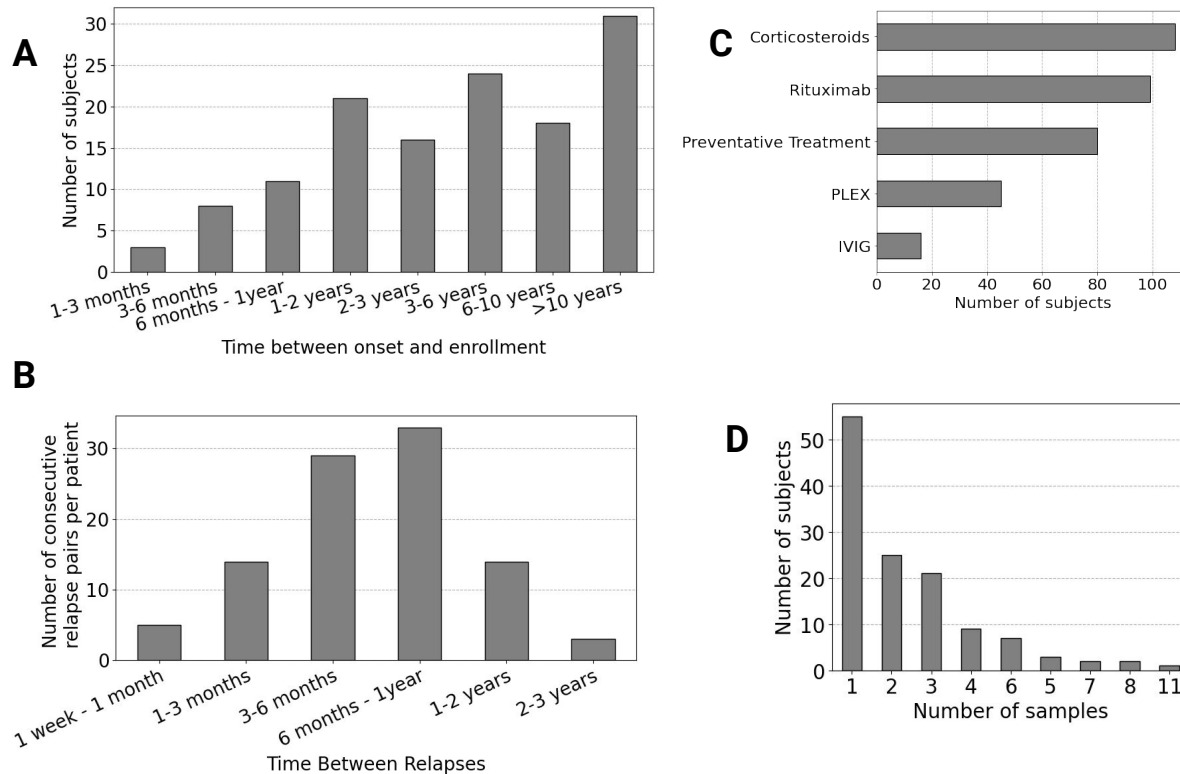


Figure 1. Longitudinal cohort characteristics. **A)** Distribution of times between onset and enrollment, computed on the full cohort. **B)** Distribution of the time between relapses, computed on the subset of the cohort who has more than one relapse. **C)** Number of patients who have received each treatment. **D)** Distribution of the number of samples per subject, computed on the full cohort.

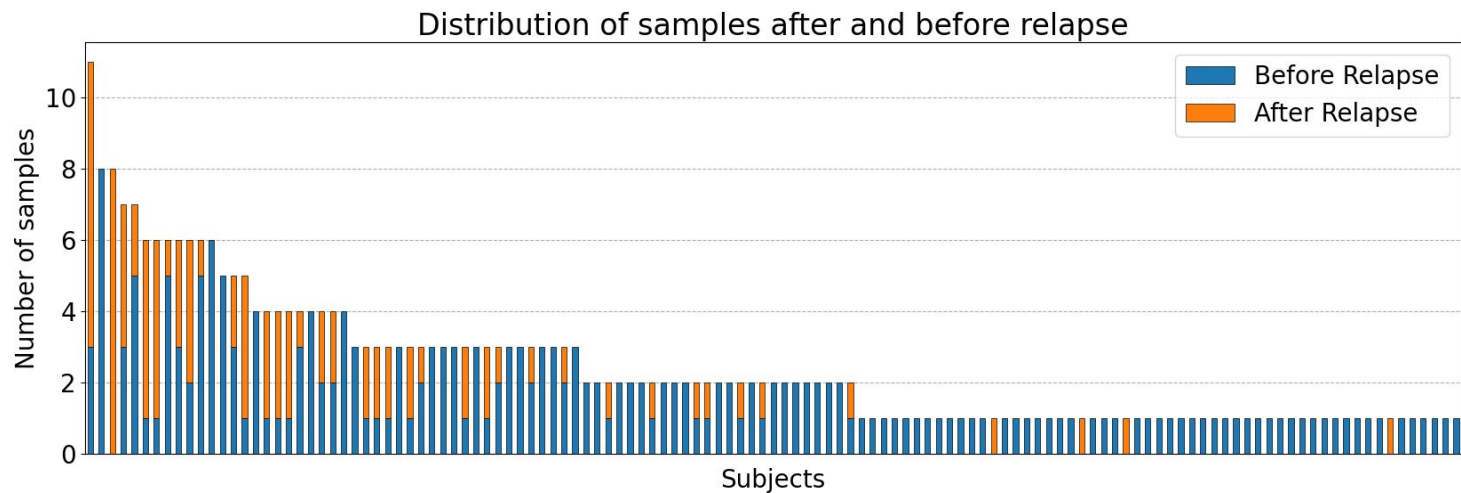
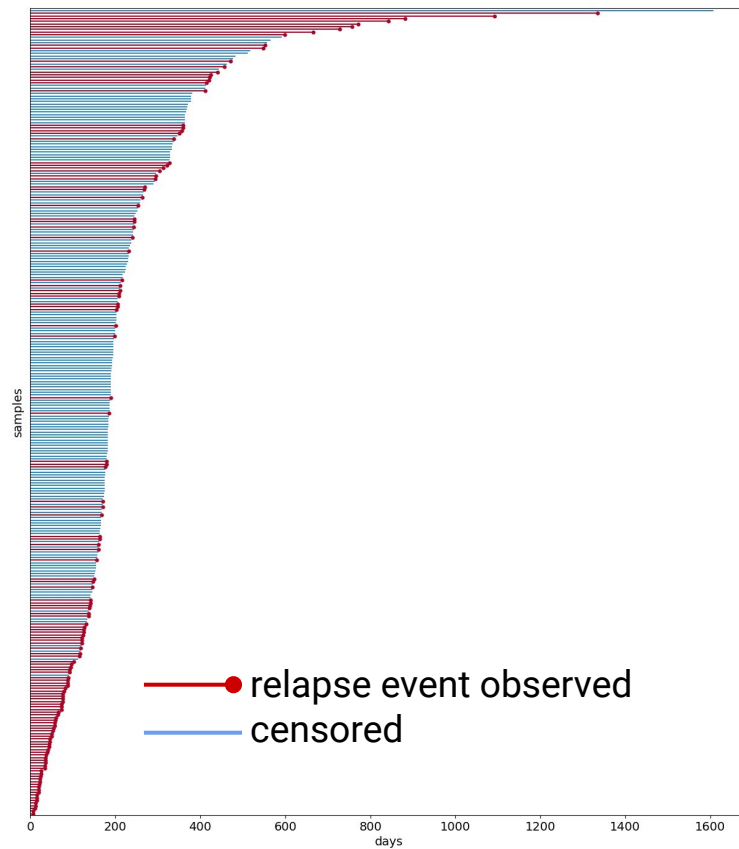


Figure S3. Temporal relationship between sample collection and relapses. Distribution of the number of samples collected after or before a relapse for all subject, computed on the full cohort. A sample collected between two relapses is classified as before a relapse in this case.

Naive Intervals

- Most intervals built with method are shorter than 1 year.
- Each interval starts when a sample is collected.
- An interval can be right-censored if the patient is lost for follow-up or if another sample was collected.
- Each sample is treated independently, even if they are derived from the same patient.



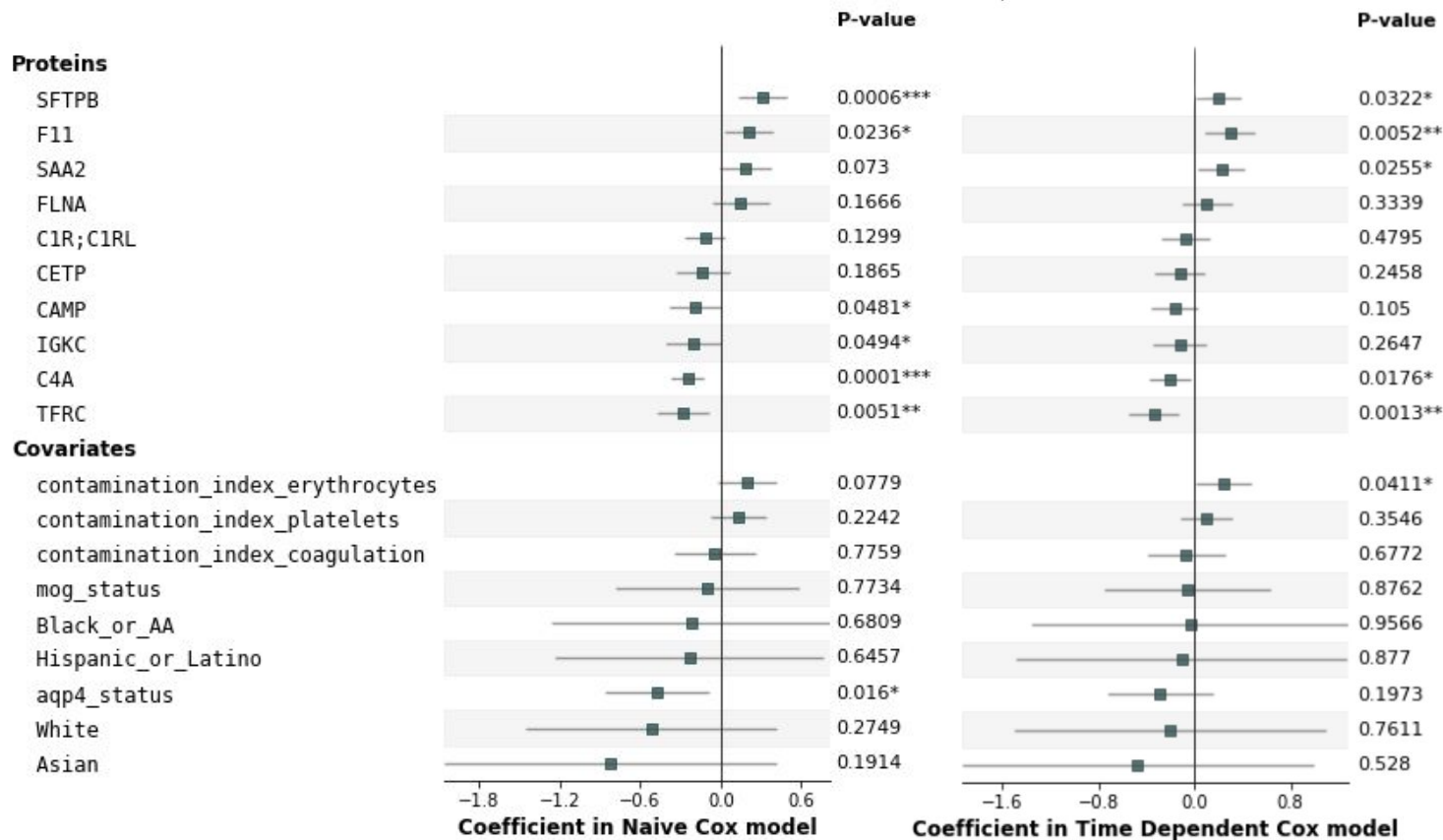


Figure S7. Summary forest plot of Naive Cox and Time Dependent Cox models fit on decaprotein signature and clinical covariates. The exponential of the coefficient for protein X can be interpreted as the hazard ratio for protein X. This measures how the risk of relapse changes for a one unit increase in the expression of protein X.

Proteomics Data Normalization

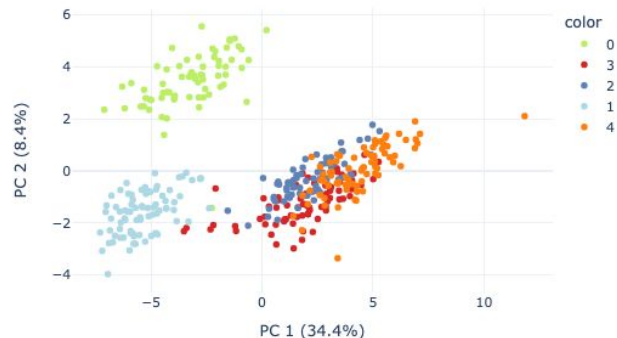
Proteomics data processed using the Compbio [proteomics pipeline](#) based on Dia-NN, **265** detected proteins.

We perform **sample level** and **protein level** normalization

:

- Sample-wise standard scaling, mean and std computed ignoring the non detected values
- Clipping values with Z-scores with absolute values above 2.5
- Setting values of non detected proteins to -2.5.
- Column-wise standard scaling

Protein level
Sample level



PCA of gene expression values, grouped by batch*

*batch 0 is the batch from phase 1 of the collaboration

Modeling 2 & 3: Survival Analysis

- It's the branch of statistics that deals with the analysis of time-to-event data (in our case the event of interest is a relapse)
- It deals with the issue of **censoring**: it is possible that not all the events have occurred during the observation period or that some subjects have been lost to follow up before experiencing the event (right censoring)
- The **Cox model** can be seen as an extension of traditional regression models that take into account censoring
- It can be evaluated using the **Concordance Index**
- Data for traditional survival analysis are in the form :

subject	duration	event
1	10	1
2	3	0
3	2	1
4	25	0

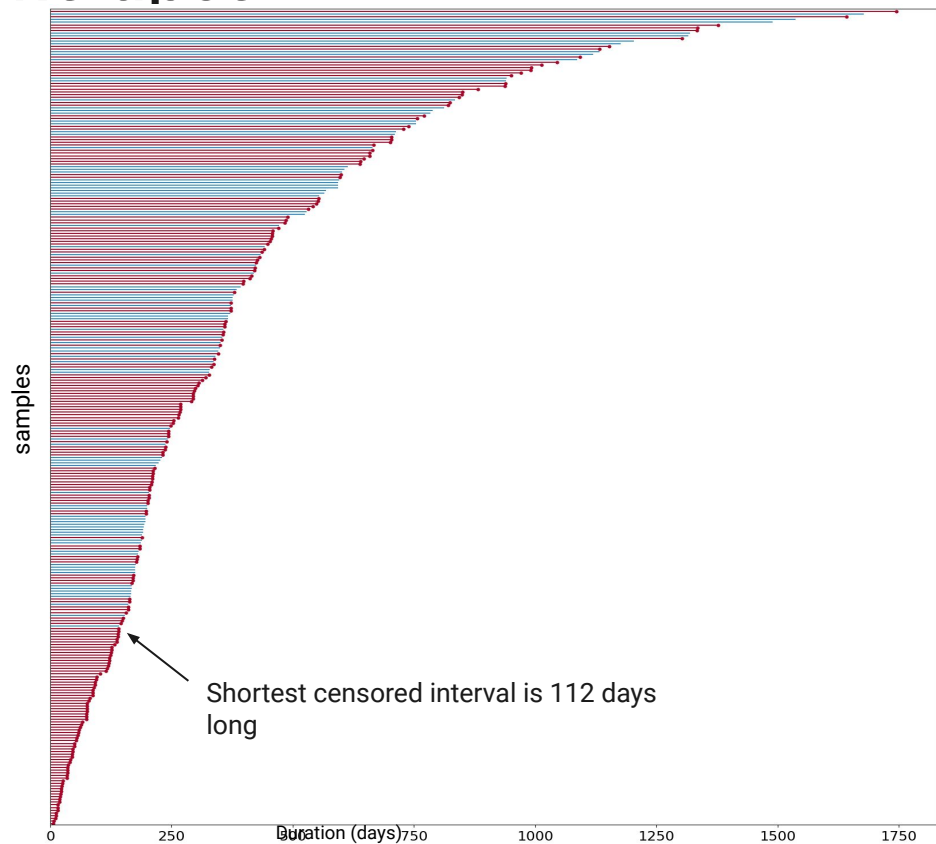
The subject had the event at time 10



The subject was censored at time 3 and the event was not observed



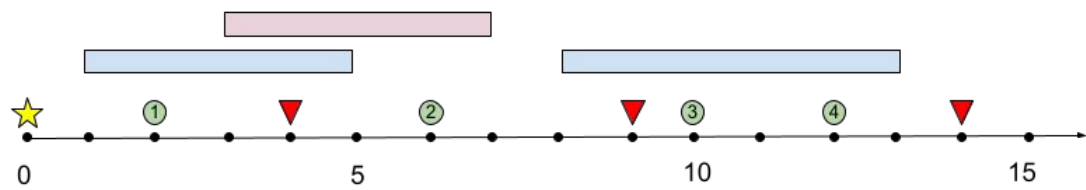
Distribution of Naive Interval Duration Censoring only by Relapse



Plot of timelines for all 305 samples

—●— : relapse event observed
— : censored

Example timeline



- ★ Study Enrollment
- Sample Collection
- ▼ Relapse
- Treatment A
- Treatment B

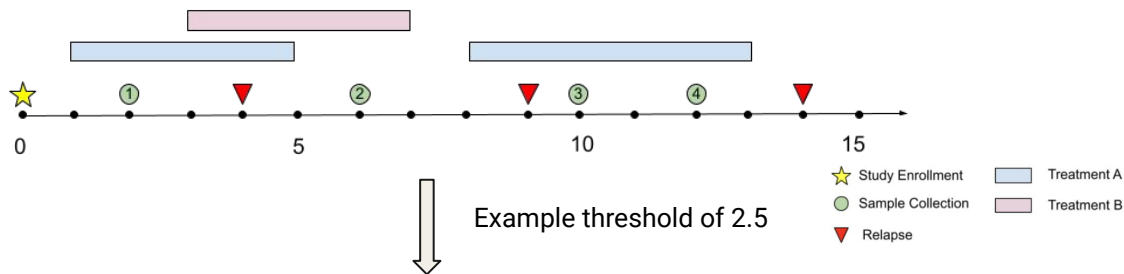
Original Data

subject_id	event_type	start_date	end_date	event_id
39	1 enrollment	0	0	
	1 treatment A	1	5	
	1 sample collection	2	2	1
	1 treatment B	3	7	
	1 relapse	4	4	
	1 relapse	4	4	
	1 sample collection	6	6	2
	1 treatment A	8	13	
	1 relapse	9	9	
	1 sample collection	10	10	3
	1 sample collection	12	12	4
	1 relapse	14	14	

Modeling 1: Classification

Assign a label to each sample : *will the patient experience a relapse in the next ... days after sample collection ?* and fit a logistic regression model.

Threshold = 120 days (~4 months) → This yields ~**20%** of positive labels.



Pros

- Easy interpretation of the model
- Easy access to a probability of relapse
- Simple dataset - one row per sample
- Can be evaluated with classification metrics

Cons

- Does not take into account treatment information
- If we increase threshold to define labels, we lose some data
- With the current threshold, unbalanced classes

subject_id	start_date	end_date	duration	sample_id	label
1	2	4	2	1	1
1	6	9	3	2	0
1	10	14	4	3	0
1	12	14	2	4	1

Cox Model

- Can be seen as an extension of traditional regression models that take into account censoring
- The log-hazard of an individual is a linear function of their covariates *and* a population-level baseline hazard that changes over time :

$$\underbrace{h(t|x)}_{\text{hazard}} = \underbrace{\widehat{b_0(t)}}_{\text{baseline hazard}} \exp \left(\underbrace{\sum_{i=1}^n b_i(x_i - \overline{x_i})}_{\text{log-partial hazard}} \right)$$

partial hazard

- Proportional Hazards Assumption : the hazard ratio is constant, the effects of the covariates do not change over time
- We can add **L1 or L2 regularization terms**

Concordance Index

- Metric used to evaluate survival models, can be seen as a generalization of the AUC
- Represents a model's ability to correctly provide a reliable ranking of survival times based on individual risk scores.

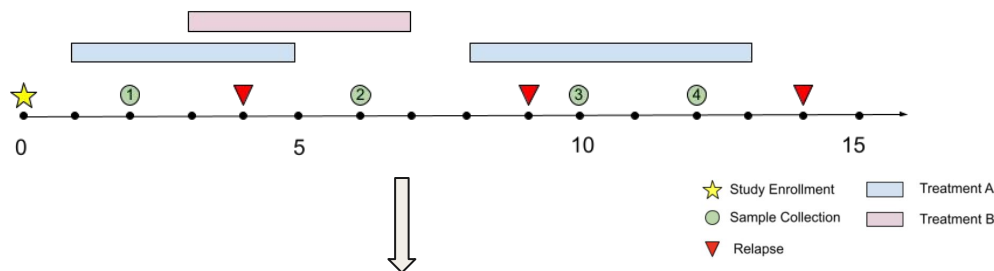
We loop over all possible pairs of subjects, for each subject i and j , we look at their risk scores r_i and r_j and their time to event T_i and T_j .

- If both i and j are not censored, then i and j are **concordant** if $r_i < r_j$ and $T_i > T_j$ (the subject with the highest risk of the event indeed experiences the event first), they are **discordant** otherwise.
- If both i and j are censored, we disregard the pair as there is no way to know who had the event first
- If j is censored and i is not:
 - If $T_i > T_j$ there is no way to know who had the event first, we disregard the pair
 - If $T_i < T_j$, we know that i had the event first, then the pair is **concordant** if $r_i > r_j$ and discordant otherwise.

$$\text{C index} = \frac{\#(\text{concordant pairs})}{\#(\text{concordant pairs}) + \#(\text{discordant pairs})}$$

Modeling 2: Naive Survival Analysis intervals

Assigns each sample to its next relapse/censoring event if censored. Fit a Cox Model PH to this survival data.



subject_id	start_date	end_date	duration	event	sample_id
1	2	4	2	1	1
1	6	9	3	1	2
1	10	14	4	1	3
1	12	14	2	1	4

Pros

- Easy interpretation of the model
- Takes into account precise interval durations
- Takes into account censoring events
- Simple dataset - one row per sample
- Can be evaluated with the C-index

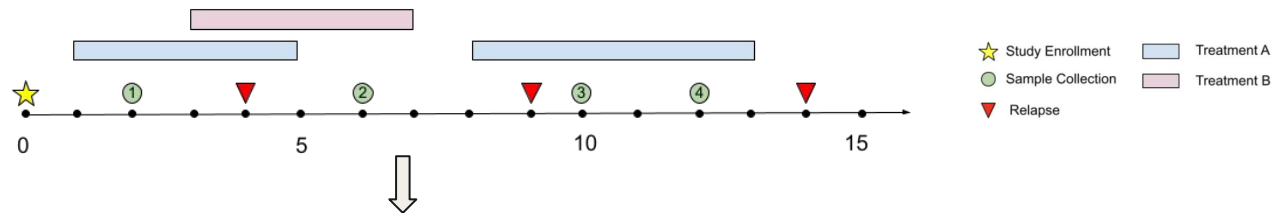
Cons

- Does not take into account treatment information
- Bias can be introduced by the fact that some time-points are counted 'twice' (ex : time interval from 12 to timepoint 14)
- We consider each interval independently

Modeling 3: Time Dependent Intervals splitting rules

- We break the timeline into **non-overlapping intervals**.
- Each time-varying covariate change and relapse triggers the start of a new interval and the end of the previous one.
 - For the time varying covariates that did not trigger the split, previous value carried forward.
 - The covariate(s) that triggered the split will have their value change to the new value
 - If a relapse event triggered the split, the event field of the previous interval will be set to 1 and a new interval is started with all other previous values of the covariates.

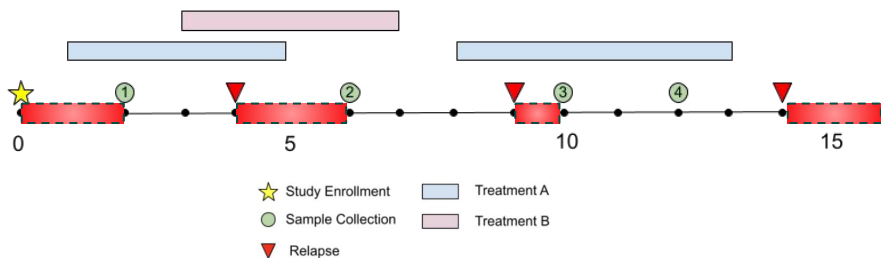
Time Dependent Intervals



subject_id	start (enrollment = 0)	stop (last known date = 15)	split_type	event	sample	treatment A	treatment B
1	0		1 treatment A : start	0		0	0
1	1		2 sample collection	0		1	0
1	2		3 treat B : start	0	1	1	0
1	3		4 relapse	1	1	1	1
1	4		5 treatment A : end	0	1	1	1
1	5		6 sample collection	0	1	0	1
1	6		7 treatment B : end	0	2	0	1
1	7		8 treatment A : start	0	2	0	0
1	8		9 relapse	1	2	1	0
1	9		10 sample collection	0	2	1	0
1	10		12 sample collection	0	3	1	0
1	12		13 treatment A : end	0	4	1	0
1	13		14 relapse	1	4	0	0
1	14		15	0	4	0	0

Time Dependent Intervals Exclusion Criteria

- We exclude intervals that do not have proteomics data associated with them, i.e. the intervals that are before the first proteomics data collection.
- We exclude intervals after a relapse and before the next sample collection → previous proteomics data collected before a relapse will likely not be representative of the protein expression state right after the relapse



subject_id	start (enrollment = 0)	stop (last known date = 15)	split_type	event	sample	treatment A	treatment B
			treatment A : start				
1	0	1	start	0		0	0
1	1	2	sample collection	0		1	0
1	2	3	treat B : start	0	1	1	1
1	3	4	relapse	1	1	1	1
1	4	5	treatment A : end	0	1	1	1
1	5	6	sample collection	0	1	0	1
1	6	7	treatment B : end	0	2	0	1
			treatment A : start				
1	7	8	start	0	2	0	0
1	8	9	relapse	1	2	1	0
1	9	10	sample collection	0	2	1	0
1	10	12	sample collection	0	3	1	0
1	12	13	treatment A : end	0	4	1	0
1	13	14	relapse	1	4	0	0
1	14	15		0	4	0	0