Università di Roma Tor Vergata

14TH EUROPEAN MEETING ON
HIV&HEPATITIS
TREATMENT STRATEGIES & ANTIVIRAL DRUG RESISTANCE
ROME, ITALY • 25 - 27 MAY 2016

Immunosuppression-driven HBV Reactivation in Patients with Resolved HBV Infection Correlates with a Relevant Risk of Evolution Towards Active Chronic Infection and Death

R. Salpini, **A. Battisti**, L. Colagrossi, C. Alteri, M. Pollicita, A. Ricciardi, C. Cerva, G. Maffongelli, M. Lichtner, C. Mastroianni, K. Casinelli, M. Paoloni, M. Marignani, S. Maylin, C. Delaugerre, F. Morisco, N. Coppola, A. Marrone, A. Brega, S. Francioso, A. Venditti, T. Mari, E. Mazzoni, N. Iapadre, D. Di Paolo, C. Sarrecchia, L. Sarmati, M. Andreoni, G. Taliani, M. Angelico, C.-F. Perno, V. Svicher

Abst#_O_09

The authors declare that there are not conflict of interest

BMS PARTNERING FOR CURE
HIRMA PROJECT
BANDIERA PROJECT
AVIRALIA Fondazione onlus

Background

How do we define immunosuppression-driven HBV reactivation?

HBV reactivation *is defined as:*

- a marked rise of serum HBV-DNA (> 2 log IU/ml from baseline level) in patients with chronic HBV infection (both in the active and in the inactive form)

or

- a reappearance of serum HBV-DNA (>100 IU/ml) in patients with apparently resolved HBV infection during or after the administration of immunosuppressive therapy

Hwang & Lok, Nat Rev Gastroenterol Hepatol. 2014

Background

Clinical manifestations of HBV reactivation according to pre-reactivation serological status

Active carrier (defective immunological control)	***Hepatitis exacerbation*** (progression of liver disease)
Inactive carrier (partial immunological control) **HBsAg- and anti-HBc+ subject** (optimal immunological control)	***Hepatitis B reactivation*** (from transient liver damage up to fulminant hepatitis)

Aim of the study

To provide a snapshot of virological and clinical features of patients, undergoing HBV-reactivation driven by immunosuppressive-therapy with a focus on reactivated patients with apparently resolved HBV infection

Methods

This study includes **80 patients**
with immunosuppression driven HBV-reactivation (HBV-R)
defined according to Hwang, 2014

Statistical analysis

Mann-Whitney test and **Fischer's Exact test** were used to assess statistically significant differences between **factors positively or negatively associated with HBV-R**.

Survival analysis

Kaplan-Meier analysis was used to estimate cumulative probability after HBV-reactivation of:

- transaminases normalization,
- undetectability of serum HBV-DNA,
- loss of HBsAg,
- death **(competing risk analysis)**.

Genetic analysis

Mean genetic distance was used to estimate the extent of genetic variability in HBsAg in a subset of 55 HBV-reactivated patients infected with genotype D.

Presence of **HBsAg-mutations associated with HBV-R** (Salpini, 2015) was investigated.

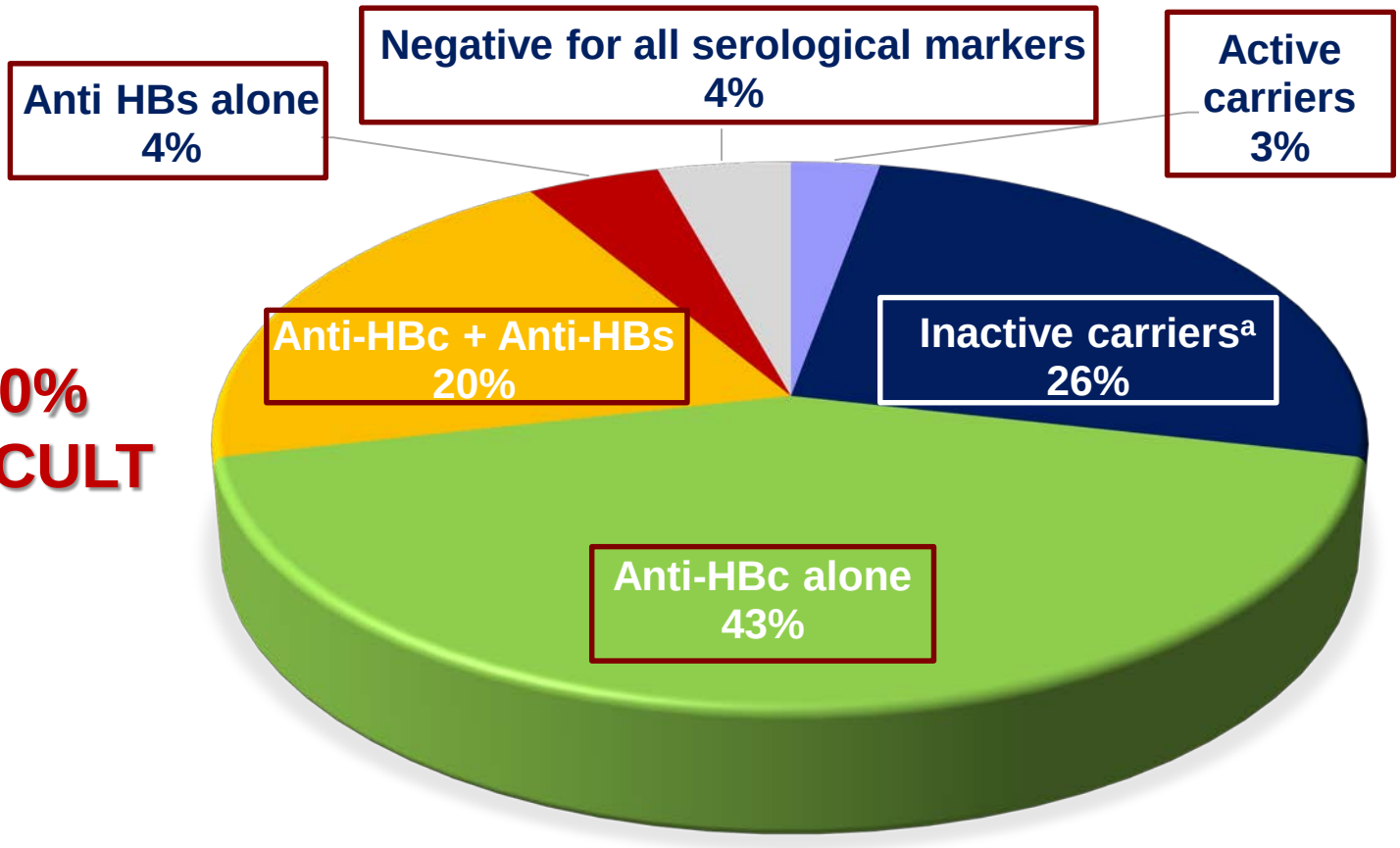
Results

Patients' characteristics at HBV-reactivation

Patients' characteristics	**N=80**
Male, N (%)	53 (66.2)
Italian nationality, N (%)	64 (80)
Median age, years (IQR)	63 (54-71)
HBV characteristics at reactivation:	
Median HBV-DNA, log IU/ml (IQR)	6.7 (4.5-7.9)
Median quantitative HBsAg, IU/mL (IQR)	8679 (1069-25776)
Median ALT, IU/L (IQR)	117 (40-621)
Median AST, IU/L (IQR)	91 (32-286)
Median MELD score (IQR)	9 (7-14)
Serological profiles at reactivation[a]:	
HBsAg positive/Anti-HBs negative N (%)	54 (69.2)
HBsAg positive/Anti-HBs positive, N(%)	**9 (11.5)**
HBsAg positive with Anti-HBs unknown, N(%)	6 (7.7)
HBsAg negative/Anti-HBs positive, N(%)	**4 (5.1)**
HBsAg negative/Anti-HBs negative, N(%)	**5 (6.5)**

[a] Datum available for 78 patients

Serological status of HBV infection at screening before starting immunosuppressive therapy



- Percentages were calculated on overall population of 80 patients. [a] Inactive carrier state was defined as HBV-DNA levels <2000 IU/ml with persistently normal transaminases.

Occult hepatitis B virus in liver tissue of individuals without hepatic disease☆

Giovanni Raimondo[1,*], Giuseppe Navarra[2], Stefania Mondello[1], Lucy Costantino[1], Guido Colloredo[3,†], Eugenio Cucinotta[4], Gaetano Di Vita[5], Claudio Scisca[4], Giovanni Squadrito[1], Teresa Pollicino[1]

16 individuals
HBsAg - and Anti-HBc+

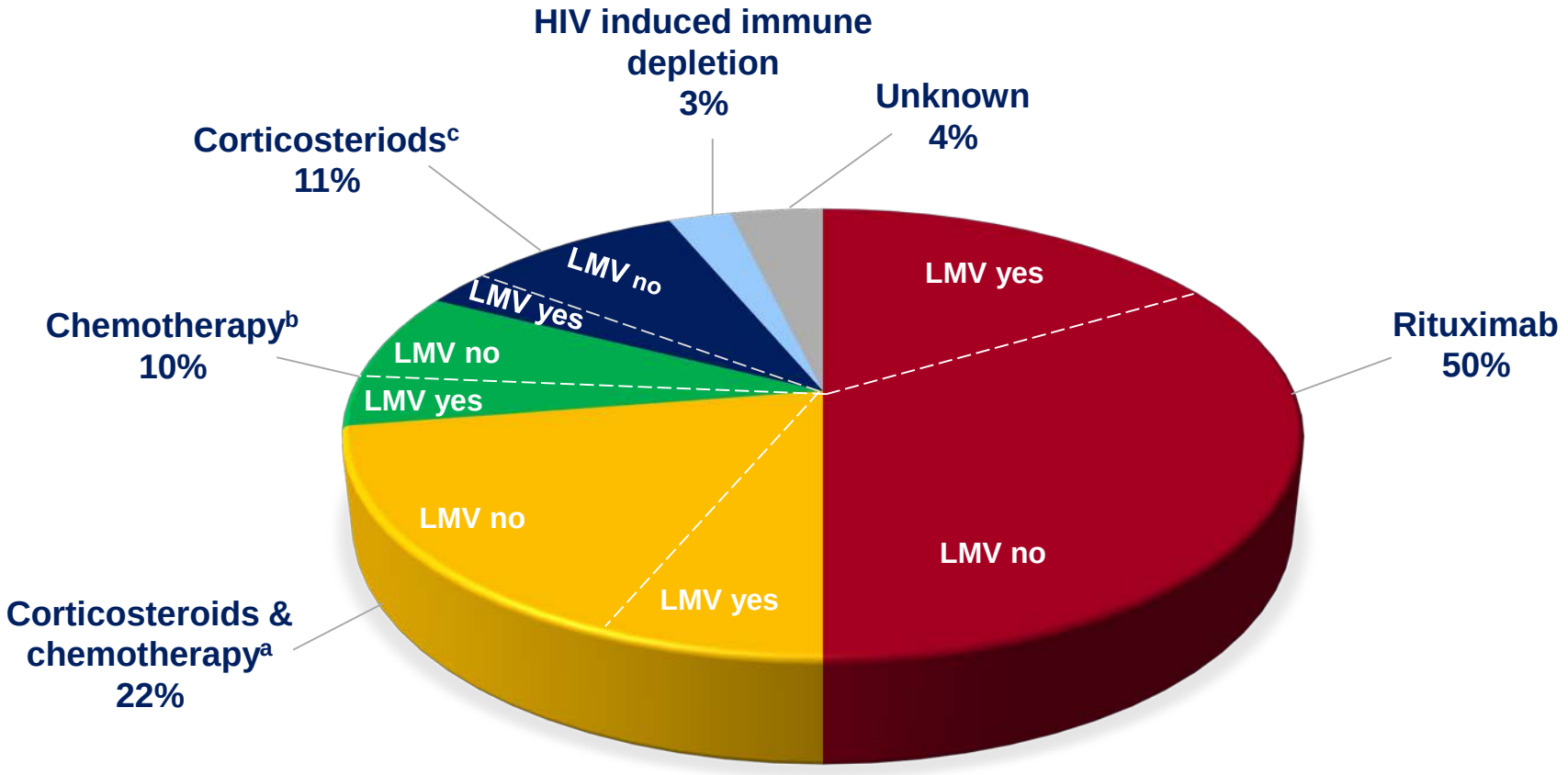
62.3% Anti-HBc positive with occult infection

82 individuals
negative for all HBV markers without a clinical history of liver disease

7.3% HBV-seronegative individuals with occult infection

"A not negligible portion of OBI cases are negative for all HBV serum markers : they might have either progressively lost the anti-HBV antibodies or might be HBV antibody negative since the beginning, as a consequence of a very limited number of virions in the infecting inoculums"

Immunosuppressive conditions associated with HBV-reactivation

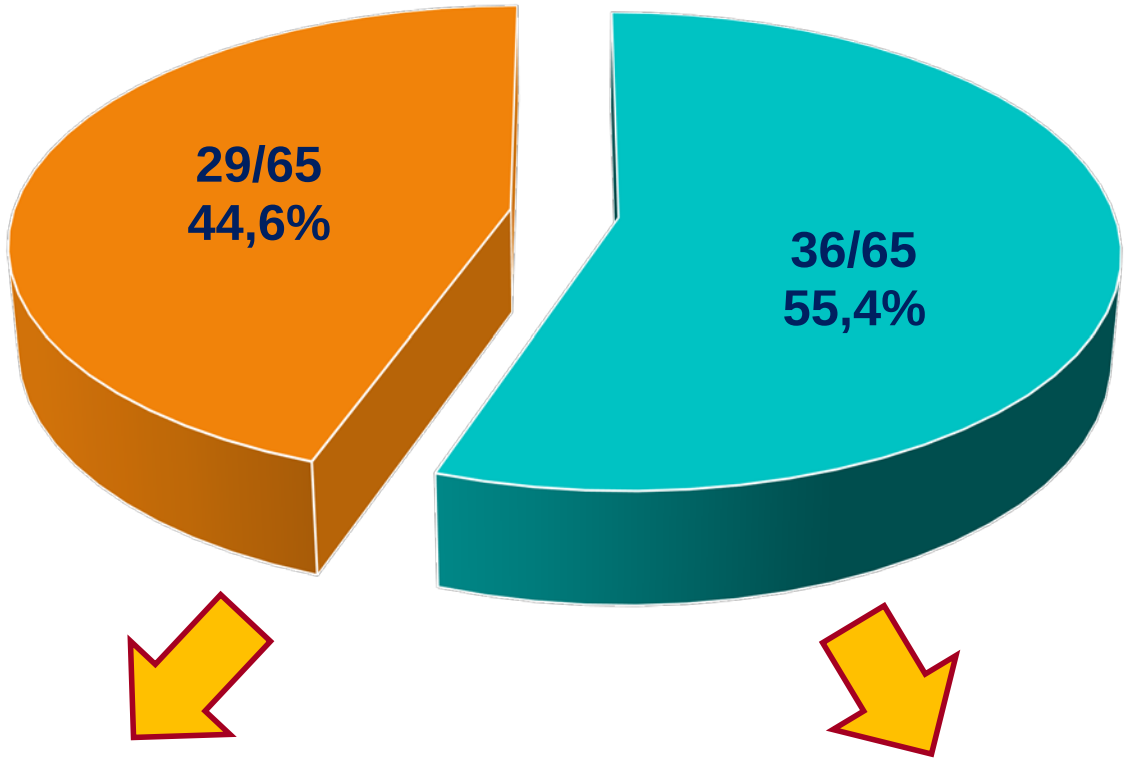


[a] Treatments with corticosteroids and chemotherapies include: methotrexate+methylprednisolone, vincristine+dexamethasone, dexamethasone + thalidomide, chlorambucil+prednisone.

[b] Treatments with only chemotherapeutics include: fludarabin, carboplatin, radiotherapy, everolimus, mycophenolate.

[c] Treatment with corticosteroids include: prednisone, deltacortene, methylprednisolone. Median (IQR) dosage of corticosteroids, mg: 5 (4-25). The duration of corticosteroids therapy ranges from 3-36 months.

In our population, a large fraction of patients develops HBV reactivation after completing immunosuppressive therapy



HBV reactivation during immunosuppression

HBV reactivation after completing immunosuppressive therapy

- Datum available for 65 patients.

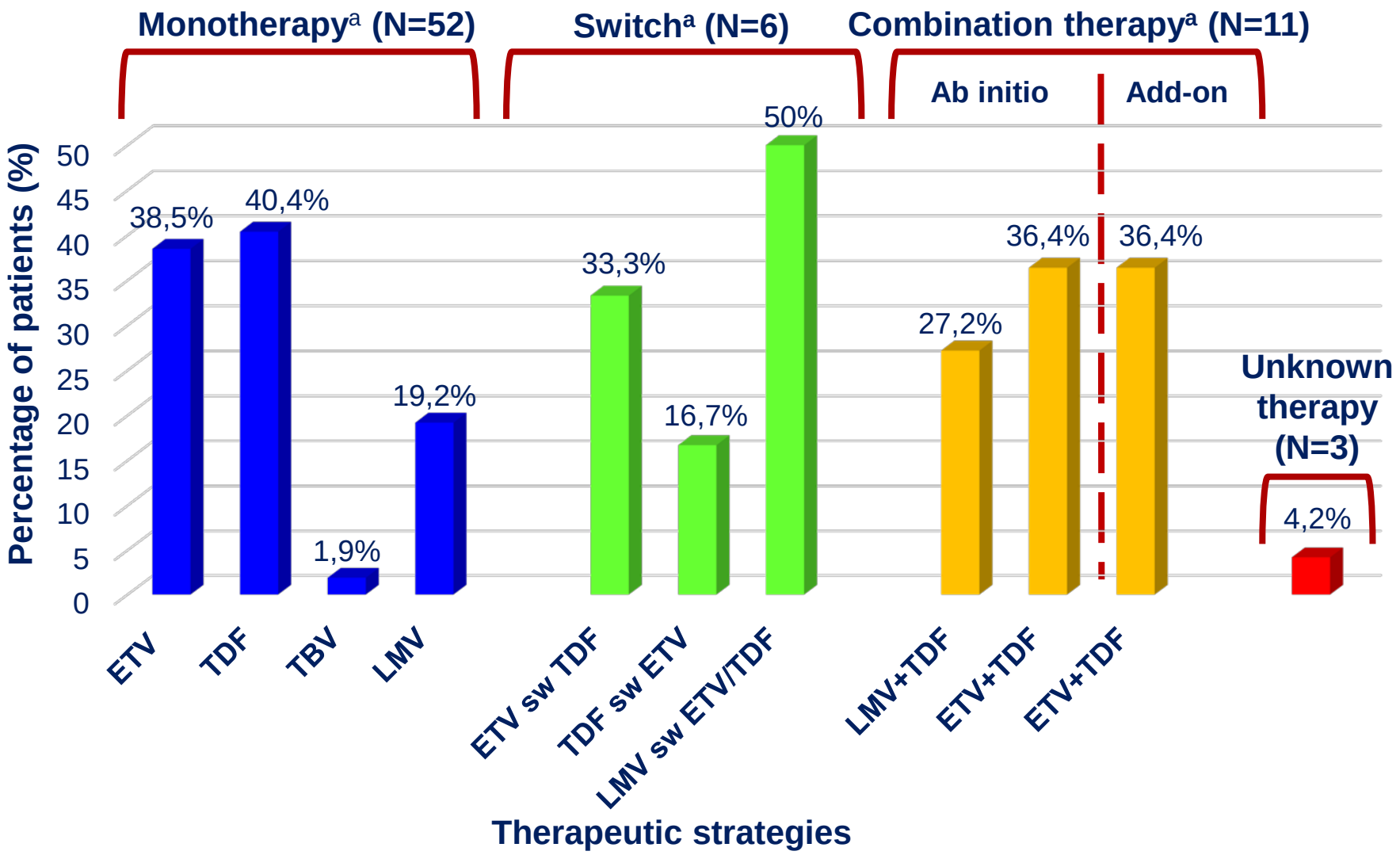
Factors **positively or negatively** associated with HBV-reactivation after completing immunosuppressive therapy

Patients' characteristics at reactivation	*HBV reactivation*		
	***DURING** Immunosuppressive therapy (N=29)*	***AFTER** Immunosuppressive therapy (N=36)*	***P** value[a]*
Median age, years (IQR)	60 (50-64)	**64 (59-73)**	**0.01**
HBV characteristics at reactivation:			
Median quantitative HBsAg, mIU/mL (IQR)	1135 (91-12871)	**16526 (1553-32972)**	**0.03**
Median AST, IU/L (IQR)	43 (31-153)	**138 (39-420)**	**0.05**
Immunesuppressive therapy:			
Corticosteroids, N(%)	**6 (21)**	1 (2)	**0.04**
Pathology requiring immune-suppressive therapy:			
Onco-hematological disease[b], N(%)	20 (69)	**33 (92)**	**0.03**

[a] Statistically significant differences were assessed by Mann-Whitney Test and by Fisher's exact test.
[b] Onco-hematological disease: chronic lymphocytic leukaemia (LLC), multiple myeloma (MM), mucosa-associated lymphoid tissue lymphoma (MALT lymphoma), bone marrow aplasia.

- The following variables were considered for the analysis: age; sex; HBsAg levels; HBV-DNA; ALT; AST; MELD; HBV serological profiles before reactivation; lamivudine prophylaxis; immunosuppressive therapies; pathologies requiring immunosuppressive therapy; exitus.

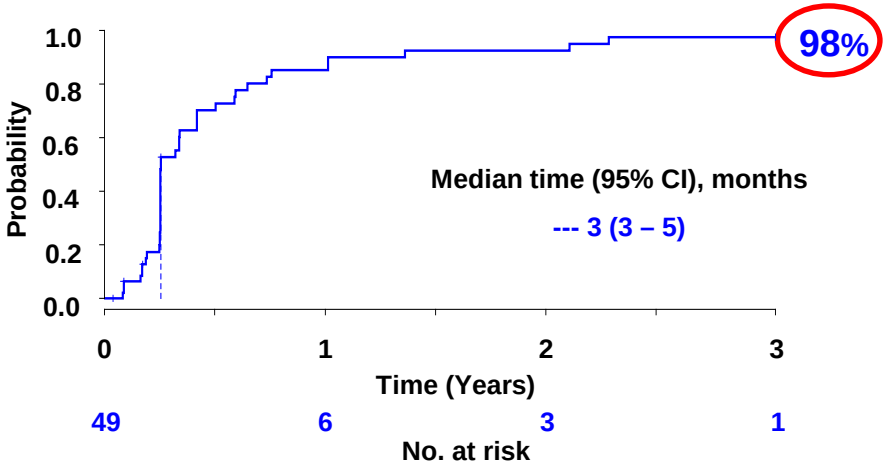
After HBV-reactivation, most patients were treated with high genetic barrier drugs with a median time of follow-up (IQR) of 30 (14-46) months



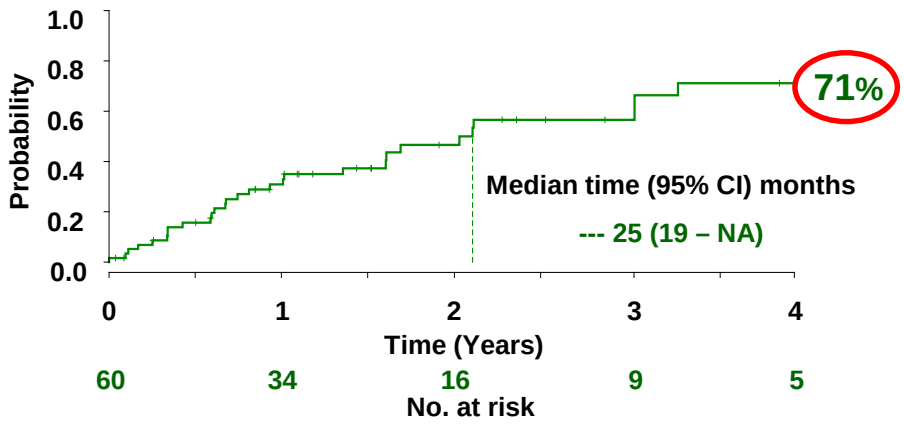
[a] Datum available for N=72 patients.

Despite ALT normalization and HBV-DNA undetectability HBsAg loss is observe in only 34%

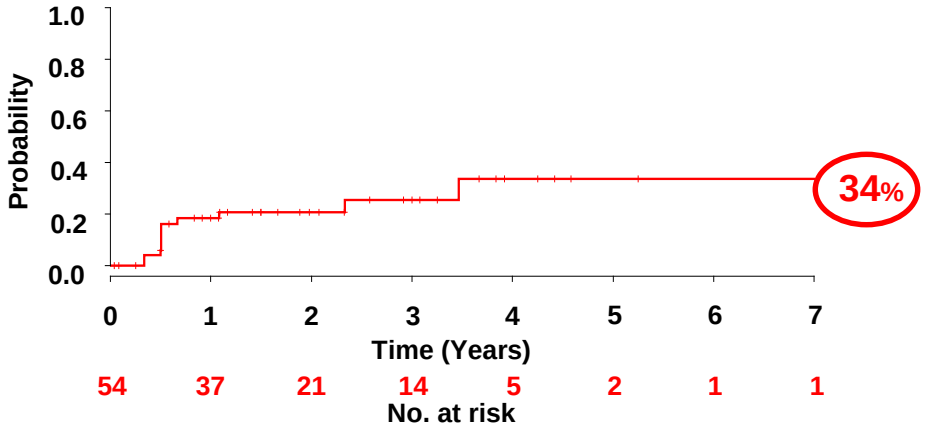
Cumulative probability of ALT normalization



Cumulative probability of undetectability of HBV-DNA



Cumulative probability of HBsAg loss



- **Kaplan-Meier analysis** was used to estimate cumulative probability after HBV-reactivation of achieving transaminases normalization, undetectability of serum HBV-DNA, loss of HBsAg **after starting anti-HBV therapy.** Patients were followed from the date of HBV-reactivation.

CI: confidence interval. NA: not available.

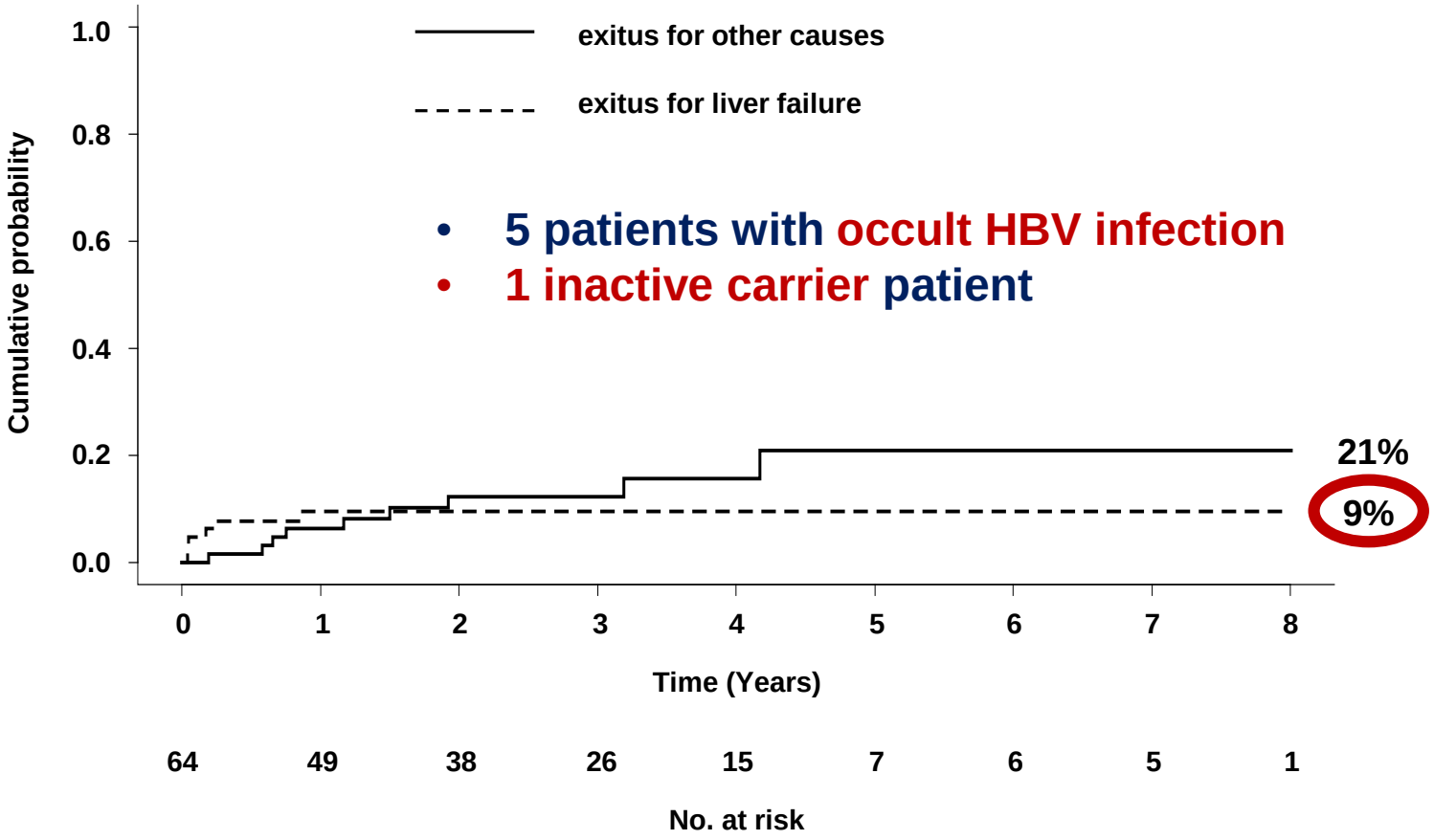
Among patients with a past occult infection (HBsAg neg, N=35) only 31.4% of patients returns to the pre-reactivation status, after a median time of 3.5 years

N of patients	35
Duration of treatment median (IQR), months	41 (IQR: 25-84) (min-max:9-95)
Transaminases normalization N(%)	26 (74.3%)
Undetectable serum HBV-DNA N(%)	15 (42.9%)
Still HBsAg positive	**24 (68.6%)**
HBsAg-loss	**11 (31.4%)**

All patients had a pre-serological status compatible with occult HBV infection, and were treated with TDF and/or ETV at the time of HBV reactivation.

Chronicization of HBV infection after reactivation, requiring long-term (life-time) antiviral treatment

In our cohort of 67 patients developing HBV reactivation, **8.9% (6/67)** die for hepatic failure related to HBV reactivation:



- **Competing risk analysis** was used to estimate the cumulative probability of exitus. Cumulative probability was evaluated in 64 patients with follow up and (when occured) date of exitus available.

Methods

This study includes **80 patients**
with immunosuppression driven HBV-reactivation (HBV-R)
defined according to Hwang, 2014

Statistical analysis

Mann-Whitney test and **Fischer's Exact test** were used to assess statistically significant differences between **factors positively or negatively associated with HBV-reactivation**.

Survival analysis

Kaplan-Meier analysis was used to estimate cumulative probability after HBV-reactivation of:

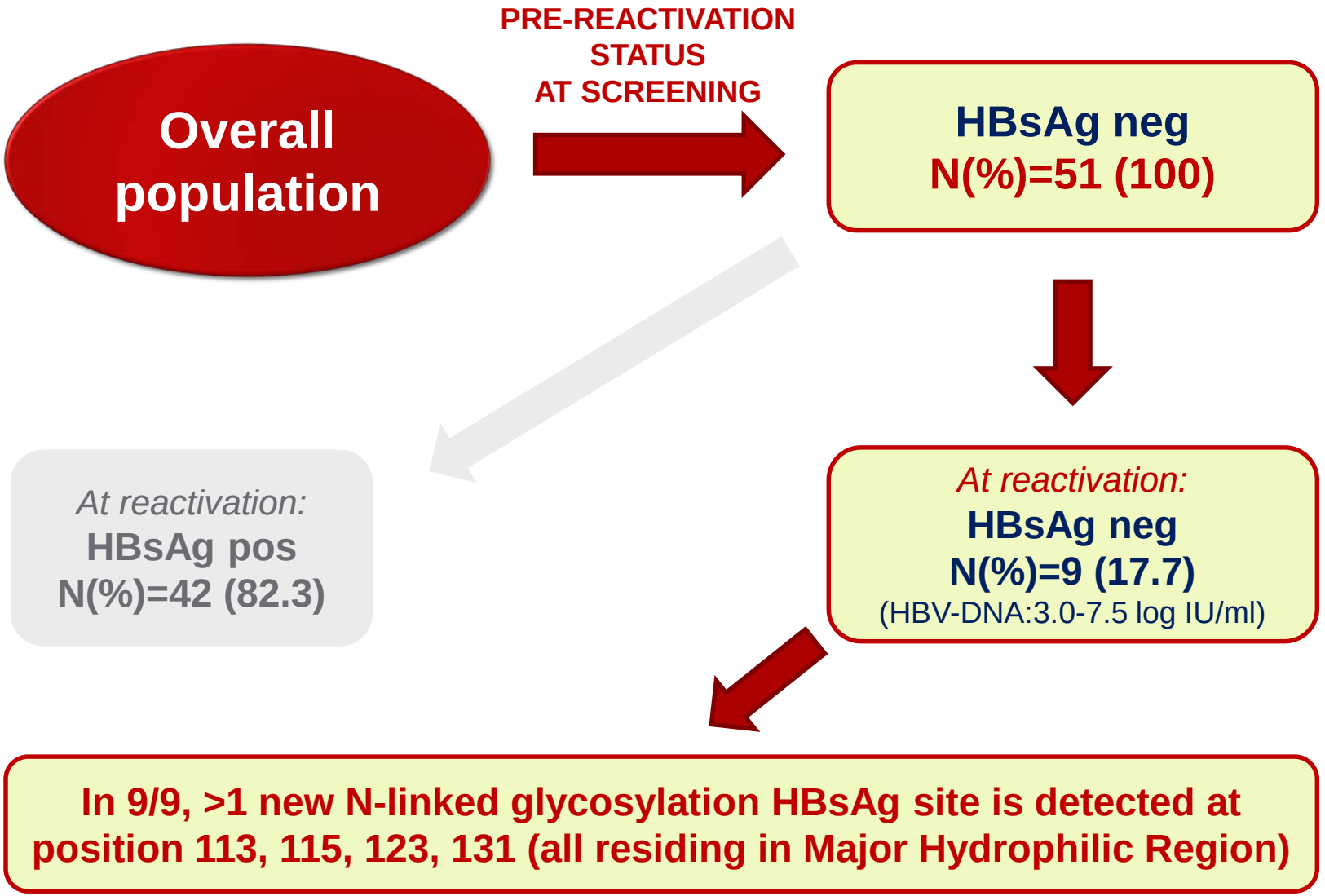
- transaminases normalization,
- undetectability of serum HBV-DNA,
- loss of HBsAg,
- death **(competing risk analysis)**.

Genetic analysis

Mean genetic distance was used to estimate the extent of genetic variability in HBsAg in a subset of 55 HBV-reactivated patients infected with genotype D.

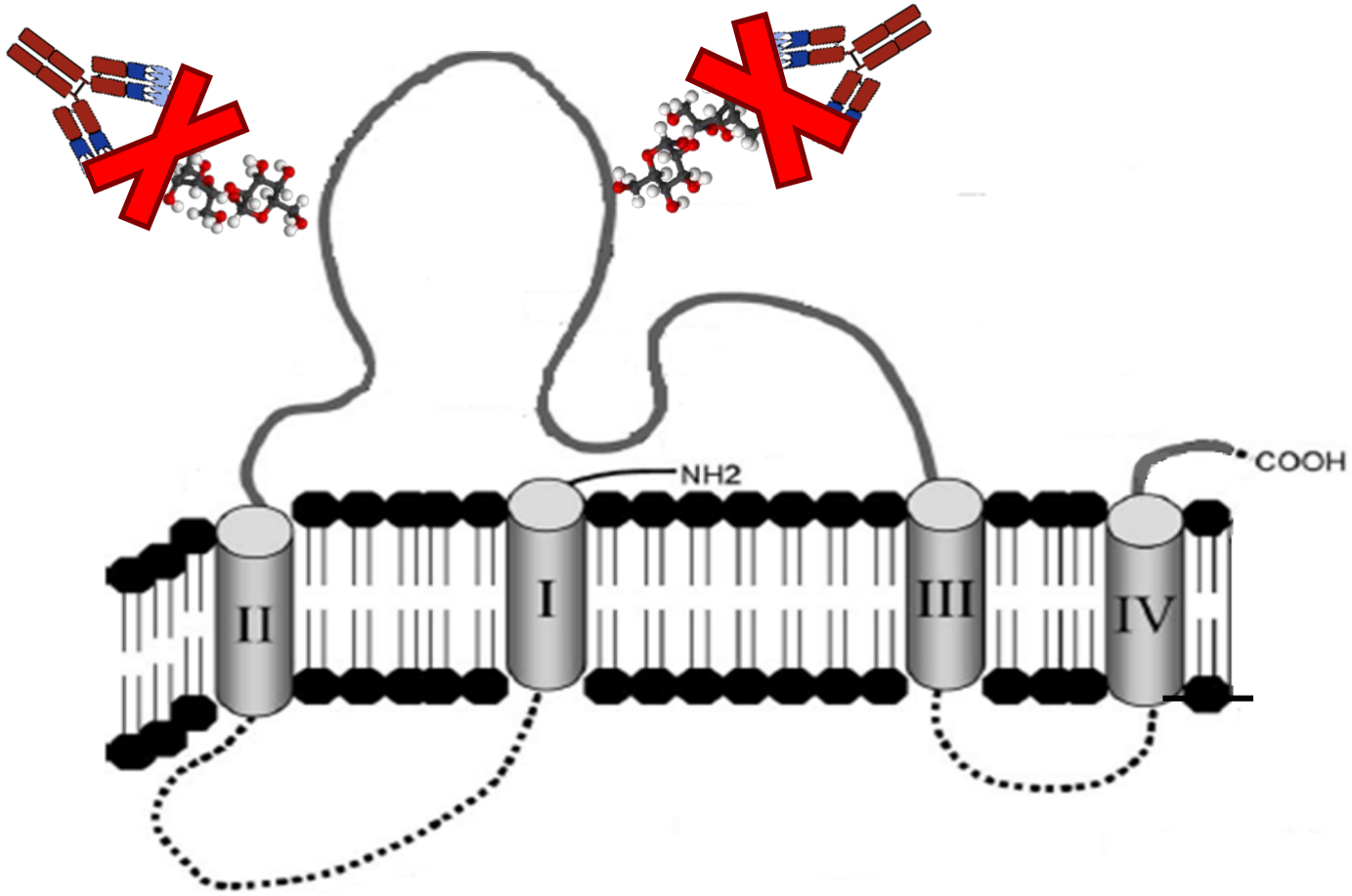
Presence of **HBsAg-mutations associated with HBV-R** (Salpini, 2015) was investigated.

Of 51 patients negative to HBsAg at screening before immunosuppression, 17.7% (9/51) patients remains HBsAg-negative despite HBV-reactivation

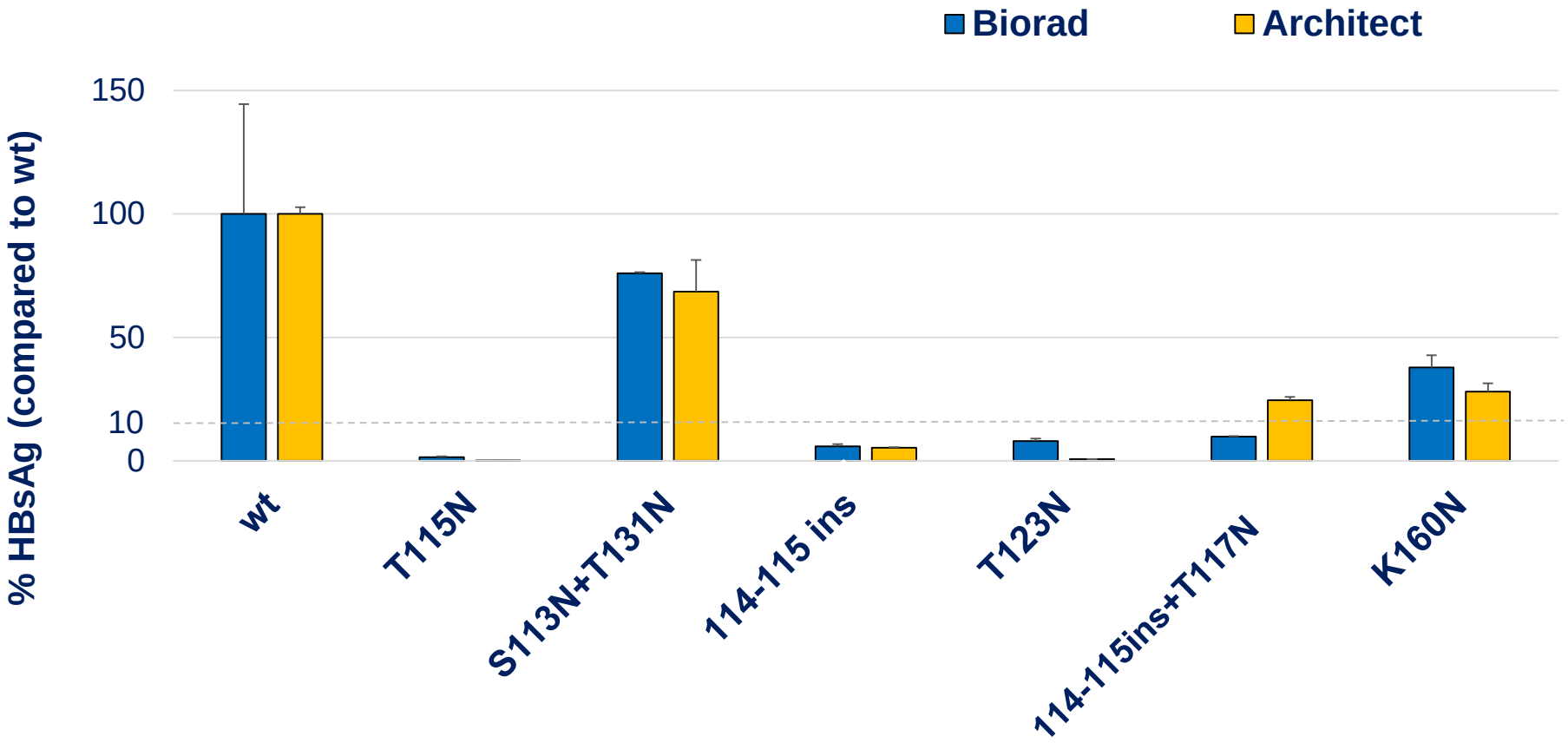


Additional N-linked glycosylation sites are anchors for glycan attachment

The hyperglycosylation of HBsAg might mask HBsAg epitopes interfering with its recognition by immunity and diagnostic antibodies (anti-HBs)



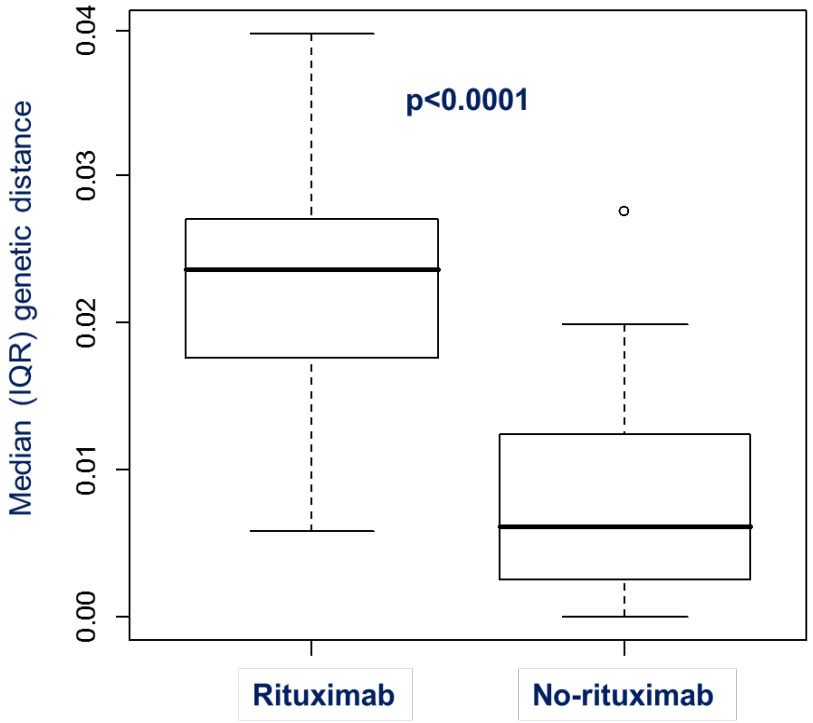
N-Glycosylation mutations strongly affects HBsAg recognition and quantification by diagnostic tests

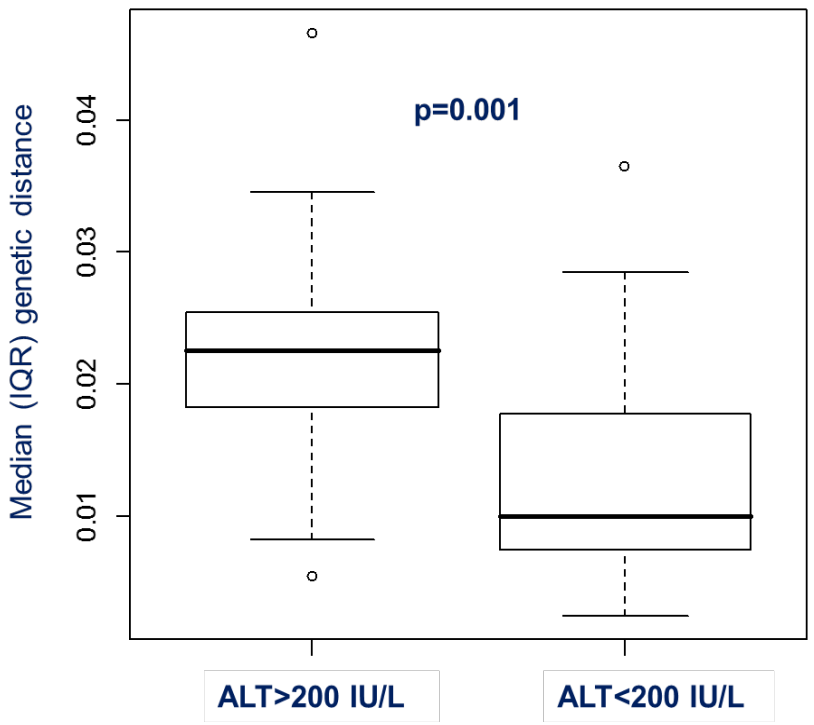


The histogram reports the **quantification of strep-tagged HBsAg** released in supernatants of HepG2 cell cultures **by different ELISAs** are shown. For each mutant, the amount of strep-tagged HBsAg released in supernatants was expressed as a percentage, considering the amount of the WT strep-tagged HBsAg as 100%. Dotted line indicates a 90% inhibition in HBsAg recognition and quantification.

Salpini et al., Hepatology 2015; Colagrossi et al., AISF 2015

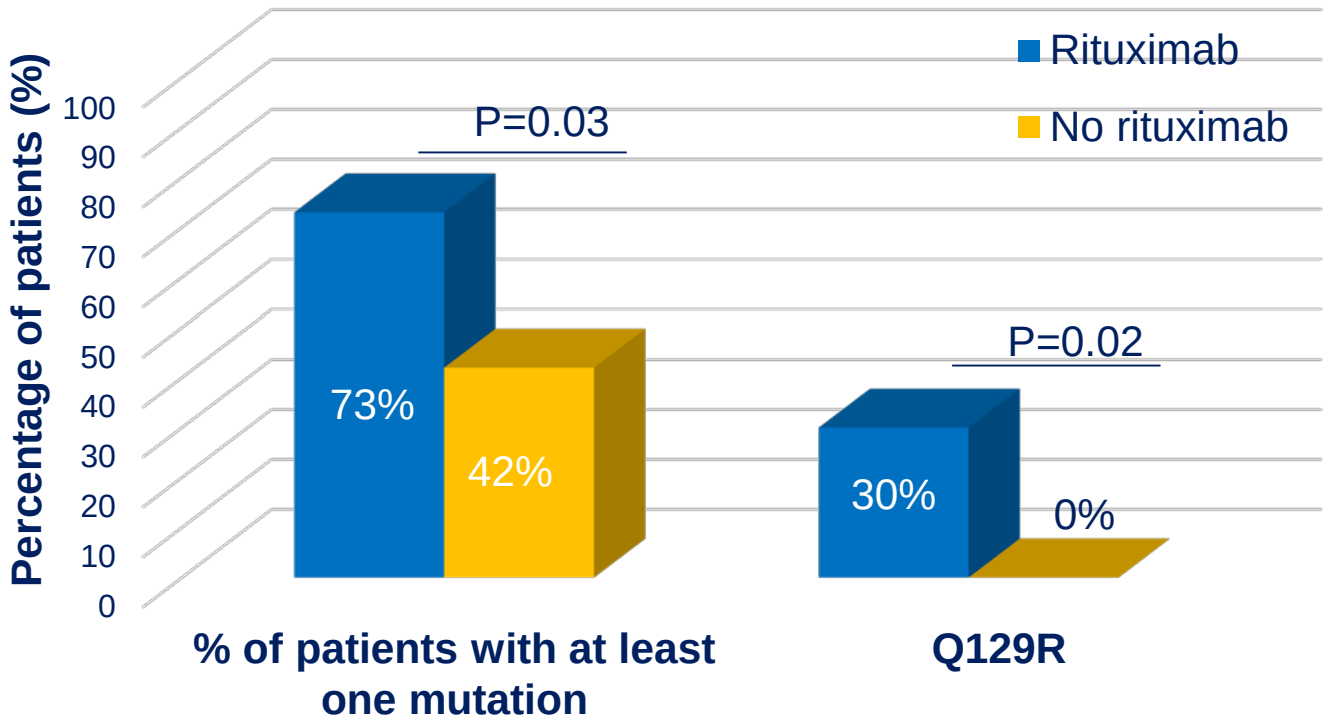
An higher degree of genetic variability in HBsAg correlates with rituximab-related immunosuppression and higher ALT levels





- Genetic distance was estimated as the extent of nucleotide substitutions per site determined by the Tajima-Nei model of MEGAv5.
- Box plots were used to report the distribution of GD values. Box plots report median, 25th percentile, 75th percentile, lower and upper whiskers, minimum and maximum values.
- Statistically significant differences were assessed by Mann-Whitney Test.

Q129R correlates with rituximab-related immunosuppression, suggesting a role in promoting HBV-reactivation in the setting of B-cell depleting drugs



- The following mutations were analyzed: C48G, V96A, Y100S, M103I, M103T, L109I, T118K, P120A, Q129H, Q129R, Y134H, S143L, D144E, G145A, G145R, S154P, E164D, S171F, L175S, Q181R, G185E.
- Statistically significant differences were assessed by Fisher Exact Test.

Conclusions

- Immunosuppression- driven HBV reactivation, can occur in a large variety of anti-HBV serological profiles and immunosuppressive settings.
- A relevant proportion of patients remains **HBsAg-negative despite HBV-reactivation**, **highlighting the importance of HBV-DNA (more than HBsAg) in HBV-reactivation diagnosis.**
- **A higher degree of genetic variability and specific mutations in HBsAg**, such as **Q129R**, **are correlated with Rituximab use and may favor HBV reactivation in the setting of drug-induced B-cell depletion.**
- Overall, these data support the **need of an optimized management of HBV-reactivation** in terms of **adequate monitoring before and during immunosuppression**, and **improved prophylaxis**.

Thanks to…

The Clinicians:

Prof. Massimo Andreoni, Loredana Sarmati, Cesare Sarrecchia, Carlotta Cerva, Alessandra Ricciardi, Gaetano Maffongelli, *Università degli studi di Roma Tor Vergata*

Prof. Mario Angelico, Arianna Brega, Simona Francioso, Daniele Di Paolo, *Università degli studi di Roma Tor Vergata*

Prof.ssa Gloria Taliani, *Policlinico Umberto I, ''Sapienza'' Università di Roma*

Nicola Coppola, Aldo Marrone, Chiara D'Amore, Isabella Siniscalchi, II *Università degli studi di Napoli*

Filomena Morisco, *Università degli studi di Napoli 'Federico II'*

Miriam Lichtner, Claudio Mastroianni,*''Sapienza'' Università di Roma*

Massimo Marignani, *Ospedale 'Sant'Andrea', Roma*

Katia Casinelli, *Ospedale di Frosinone*

Maurizio Paoloni, *Ospedale di Avezzano*

Sarah Maylin, Constance Delaugerre, *AP-HP Hopital Saint-Louis, Laboratoire de Virologie, Paris*

Ettore Mazzoni, *Policlinico Casilino, Roma*

Nerio Iapadre, *Ospedale San Salvatore, L'Aquila*

Virology Group
University of Rome Tor Vergata:

Prof. Carlo Federico Perno

Valentina Svicher

Romina Salpini

Luna Colagrossi

Claudia Alteri

Michela Pollicita

Acknowledgments:

BMS
PARTNERING FOR CURE

HIRMA
PROJECT

BANDIERA
PROJECT

AVIRALIA onlus
Fondazione