# PROTOCOL OF THE CLIgAN STUDY

**Title**: "Early start corticosteroids versus standard care (RASBs followed by corticosteroids) for proteinuria in Immunoglobulin A Nephropathy (IgAN) patients with active renal lesions identified by kidney biopsy and RASBs combined with corticosteroids versus sodium-glucose cotransporter2 inhibitor combined with RASBs for proteinuria and renal function in IgAN patients with chronic renal lesions identified by kidney biopsy. **A PROJECT FOR TWO MULTICENTRE, PROSPECTIVE OPEN LABEL RANDOMIZED CLINICAL STUDIES TO EVALUATE THE EFFECT OF TARGETED THERAPY BASED ON KIDNEY BIOPSY FINDINGS IN IgAN PATIENTS (CLIgAN)"**

**Short title:** "A project for two multicentre, prospective open label randomized clinical studies to evaluate the effect of targeted therapy based on kidney biopsy findings in IgAN patients (CLIgAN)"

**Trial Registration:** ClinicalTrials.gov NCT 04662723

**Protocol version and version date:** 4.0 – 14/Sep/2022

**Fundings:** Italian Ministry of University (PON RI No. ARS01_00876)
Drug Company: No-profit independent study
Time frame support 2020-2023.

**Roles and responsibilities:** *Names, affiliations and roles of the protocol contributors*

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Steering committee will be composed of the chiefs of the renal units.

CONFIDENTIAL

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**Protocol Title:** "Early start corticosteroids versus standard care (RASBs followed by corticosteroids) for proteinuria in Immunoglobulin A Nephropathy (IgAN) patients with active renal lesions identified by kidney biopsy and RASBs combined with corticosteroids versus sodium-glucose cotransporter2 inhibitor combined with RASBs for proteinuria and renal function in IgAN patients with chronic renal lesions identified by kidney biopsy. **A PROJECT FOR TWO MULTICENTRE, PROSPECTIVE OPEN LABEL RANDOMIZED CLINICAL STUDIES TO EVALUATE THE EFFECT OF TARGETED THERAPY BASED ON KIDNEY BIOPSY FINDINGS IN IgAN PATIENTS (CLIgAN)"**

**Approved by the following:**

**SPONSOR**

| | |
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| Prof. Francesco Paolo Schena,<br>President of Fondazione Schena<br><br>*Signature* | 20.09.2022<br>*Date* |

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| | |
|---|---|
| Dott. Antonio Nicolucci,<br>CORESEARCH, Pescara<br><br>*Signature* | 20-09-2022<br>*Date* |

**INVESTIGATOR PROTOCOL APPROVAL**

**CLIgAN**

"Early start corticosteroids versus standard care (RASBs followed by corticosteroids) for proteinuria in Immunoglobulin A Nephropathy (IgAN) patients with active renal lesions identified by kidney biopsy and RASBs combined with corticosteroids versus sodium-glucose cotransporter2 inhibitor combined with RASBs for proteinuria and renal function in IgAN patients with chronic renal lesions identified by kidney biopsy. **A PROJECT FOR TWO MULTICENTRE, PROSPECTIVE OPEN LABEL RANDOMIZED CLINICAL STUDIES TO EVALUATE THE EFFECT OF TARGETED THERAPY BASED ON KIDNEY BIOPSY FINDINGS IN IgAN PATIENTS (CLIgAN)"**

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I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to principles of Good Clinical Practices and local regulations and requirements.

Investigator:

**Name**: _____

**Signature**: _____

**Date**: _____

**SYNOPSIS**

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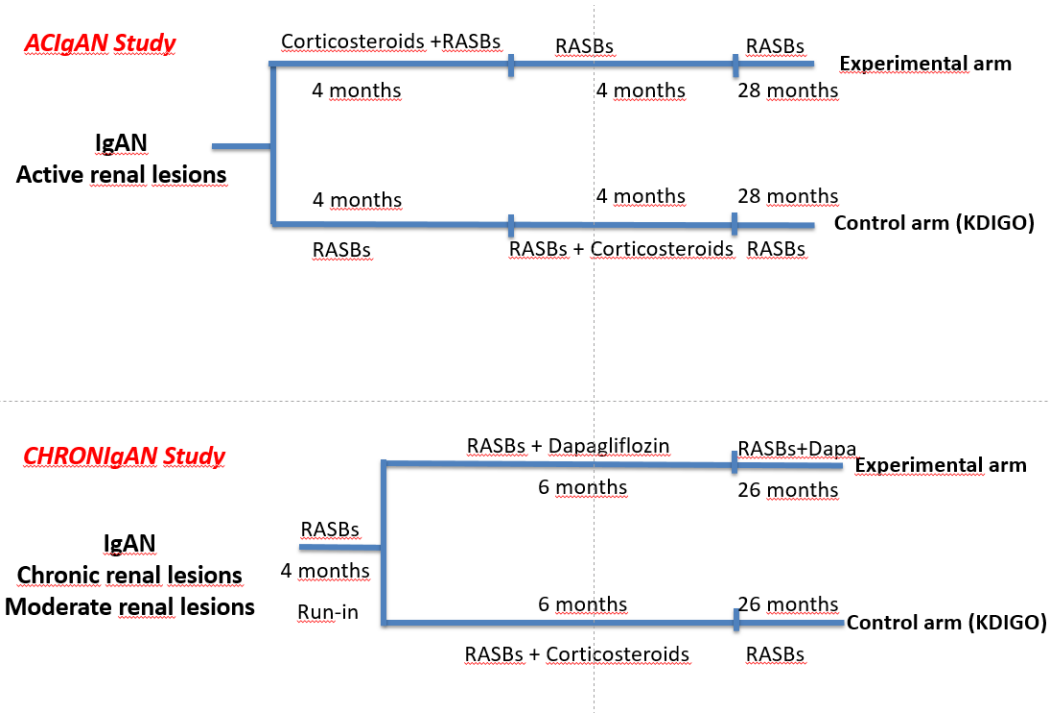
|---|---|
| **Study Title** | "Early start corticosteroids versus standard care (RASBs followed by corticosteroids) for proteinuria in Immunoglobulin A Nephropathy (IgAN) patients with active renal lesions identified by kidney biopsy and RASBs combined with corticosteroids versus sodium-glucose cotransporter2 inhibitor combined with RASBs for proteinuria and renal function in IgAN patients with chronic renal lesions identified by kidney biopsy. **A PROJECT FOR TWO MULTICENTRE, PROSPECTIVE OPEN LABEL RANDOMIZED CLINICAL STUDIES TO EVALUATE THE EFFECT OF TARGETED THERAPY BASED ON KIDNEY BIOPSY FINDINGS IN IgAN PATIENTS (CLIgAN)"** |
| **Protocol Number** | CLIgAN |
| **Study Phase** | III |
| **Study Design:** | The flow of this group of two prospective, multicentre, open-label randomized clinical trials on IgAN patients (CLIgAN) based on the kidney biopsy findings, is illustrated in the figure:<br>  <br> |
| **Target Patients** | Men and women aged 18 to 75 years with biopsy-proven IgAN will be enrolled in the CLIgAN project |

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| **Background and study rationale** | Idiopathic Immunoglobulin A nephropathy (IgAN) is the most common glomerulonephritis in the world. It is more prevalent in Asia than in Europe and the US. IgAN is estimated to affect more than 500.000 people in Europe and it is the leading nephritis in kidney-biopsied patients. Approximately 40% of IgAN patients reach end-stage kidney disease (ESKD) 20 years after their kidney biopsy. The high prevalence of ESKD shows that IgAN has a significant economic impact in the countries because renal replacement therapy is costly. Moreover, the disease's onset in the second and third decades of life represents a social challenge because patients are typically very active and highly productive in the workplace. This challenge is one more reason to move from a generalized therapy for all IgAN patients to personalized therapy.<br><br>The first edition of the KDIGO guidelines, published in 2012 and based on existing evidence from randomized controlled trials, suggested different therapeutic approaches for IgAN patients based on the clinical setting. Three sets of patients were considered:<br><br>i) *Patients with low risk* manifest minor urinary findings and proteinuria < 0.5 g/day, normal estimated glomerular filtration rate (eGFR) and normal blood pressure; they need only annual or biannual checks without therapy. They represent only 5-10% of the IgAN patient population.<br><br>ii) *Patients with intermediate risk* have proteinuria >0.5 g/day with normal or reduced eGFR and normal or high blood pressure. They may benefit from continuous supportive therapy (renin-angiotensin system blockers; RASBs) when proteinuria lowers less than 1g/day, and eGFR remains stable. When proteinuria is > 1g/day and eGFR (normal at baseline) declines to >50 ml/min/ 1.73 $m^2$, after three months of continuous supportive therapy, patients may benefit from corticosteroids combined with supportive therapy for six months. When eGFR is less than 30 ml/min or between 30 and 50 ml/min/1.73 $m^2$, only supportive treatment is recommended with no immunosuppression, except for patients with rapidly progressive glomerulonephritis.<br><br>iii) *Patients at high risk* for acute or rapid loss of eGFR caused by rapidly progressive glomerulonephritis or nephrotic syndrome may benefit from corticosteroids combined with immunosuppressive agents and supportive therapy. These patients represent 2-5% of the IgAN population. In the presence of acute kidney injury (AKI) caused by red blood cells stacked in the tubules during the macrohematuria episode, only supportive therapy is suggested. These indications have been confirmed in the recent edition of the KDIGO 2021 guidelines (5).<br><br>Unfortunately, due to the lack of experimental evidence from randomized controlled trials in this setting, the KDIGO guidelines could not consider the presence of active and chronic renal lesions at the time of the kidney biopsy as a potential *effect modifier* that could drive treatment decisions; this knowledge, nonetheless, is well recognized in clinical practice where patients with chronic renal lesions do not seem to respond to immunosuppressive treatment as opposed |

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| | to those with active renal lesions. Observational studies support these clinical findings and their number, consistency and effect sizes shown for interventions based on the findings of kidney biopsy provide equipoise for the design and conduct of randomized trials based on the specific hypothesis that response may be modified by the baseline kidney biopsy findings. Our clinical study in IgAN patients (CLIgAN) will consider, first, the type of renal lesions, and will enrol patients in the ACIgAN and CHRONIgAN study based on current recommended practice (RASB followed by steroids) versus a differential approach (early start steroids or SGLT2) based on the biopsy findings. |
| **Study objectives** | **Primary objectives:**<br>• To evaluate the therapeutic effect on proteinuria with early corticosteroid therapy, administered after the kidney biopsy in IgAN patients with active renal lesions (ACIgAN) versus standard care of RASBs alone followed by corticosteroids combined with RASBs;<br>• To evaluate the therapeutic effect of SGLT2i (Dapagliflozin) combined with RASBs versus corticosteroids combined with RASBs on proteinuria in patients with chronic or moderate renal lesions (CHRONIgAN); thus, to avoid the potential side effects of corticosteroid therapy.<br><br>**Secondary objectives:**<br>• To evaluate the effect of study treatments on the renal function and on the progression of the kidney damage in IgAN patients at 3 years<br>• after the prediction of ESKD through our IgAN CDSS tool (DialCheck) at the time of the kidney biopsy, to determine whether this personalized approach to therapy delays the impairment of the renal function. |
| **Endpoints** | **Primary endpoint:**<br>• Between-arms difference in proteinuria reduction within 4 months and in non-responders within 8 months in ACIgAN study.<br>• Between-arms difference in proteinuria reduction within 10 months in CHRONIgAN study.<br><br>**Secondary endpoints:**<br>• eGFR slope calculated as mean of individual slope obtained from individual linear regression of eGFR overtime (3 years);<br>• eGFR decline > 40 % from the baseline value;<br>• composite end point: GFR decline > 40%, ESKD (defined as long-term GFR $\leq$ 15 ml/min/1.73m$^2$ for more than three months or need for maintenance dialysis or kidney transplantation) or death due to kidney disease;<br>• absolute difference between last GFR value and baseline GFR;<br>• stable renal function defined as a decline in GFR $\leq$ 5 ml/min/1.73m$^2$ at the end of three years follow- up;<br>• mean annual change in the slope of the reciprocal of serum creatinine concentration; |

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| | • time-averaged proteinuria (TA-P) calculated as the weighted mean of all post-randomisation measurement, with weights representing the time elapsed since the previous measurement;<br>• proteinuria slope, calculated as a mean of individual slope, obtained from individual linear regression of daily proteinuria overtime (3 years);<br>• complete remission of proteinuria defined as achievement of urinary protein level ≤ 0.2 g/day or a urinary protein-to-creatinine ratio ≤ 0.2 g/g;<br>• partial remission of proteinuria defined as achievement of a urinary protein reduction ≥ 50% or greater compared with the baseline value. |
| **Number of Sites** | Over 30 |
| **Number of patients** | 432 patients:<br>• 132 patients (66 patients per group, including a 10% dropout rate, in the **ACIgAN trial)**<br>• 300 patients (150 patients per group, including a 10% dropout rate, in the **CHRONIgAN trial**) |
| **Inclusion and exclusion criteria** | **Inclusion Criteria:**<br>**<u>ACIgAN study</u>**<br>1. Males and females aged 18 to 75 years<br>2. Written informed consent form.<br>3. Biopsy-proven idiopathic IgAN with active renal lesions (E1 and/or C1) within 2 weeks.<br>4. eGFR ≥ 30 ml/min/1.73 $m^2$.<br>5. 24 hour proteinuria ≥ 0.5 g.<br>6. Patients on treatment or candidate for the treatment with RASBs (either an ACEi or ARB), as per clinical practice, according to the current KDIGO guidelines.<br><br>**<u>CHRONIgAN study</u>**<br>1. Males and females aged 18 to 75 years<br>2. Written informed consent form.<br>3. Biopsy-proven idiopathic IgAN with chronic (T1,2) or moderate (M0,1, S0,1, E0, T0, C0) renal lesions at high or very high CKD risk within 4 weeks.<br>4. eGFR ≥ 30 ml/min/1.73 $m^2$.<br>5. 24 hour proteinuria ≥ 0.5 g.<br>6. Patients on treatment or candidate for the treatment with RASBs (either an ACEi or ARB), as per clinical practice, according to the current KDIGO guidelines.<br><br>**Exclusion Criteria**:<br>1. Non biopsy-proven IgAN |

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| | 2. IgAN patients with minimal change disease at kidney biopsy and nephrotic syndrome.<br>3. IgAN patients with macrohematuria and acute renal failure.<br>4. IgAN patients with rapidly progressive glomerulonephritis (extracapillary lesions in more than 25 % of glomeruli in the kidney biopsy).<br>5. Patients with secondary IgAN (lupus nephritis, Schoenlein-Henoch purpura, liver cirrhosis).<br>6. Patients with superimposed IgAN in a kidney transplant.<br>7. Patients with other types of glomerular diseases.<br>8. Patients with solitary kidney.<br>9. Patients with end-stage kidney disease.<br>10. Bleeding disorders not responsive to treatment.<br>11. Patients with myocardial infarction or cerebrovascular stroke in the previous six months.<br>12. Severe liver diseases, infections, malignancies.<br>13. Uncontrolled diabetes (glycemia > 200 mg/dL and HbA1c > 7.5%).<br>14. Aseptic necrosis of any bone.<br>15. Any prior immunosuppressive therapy.<br>16. Other morbidities that can be exacerbated by corticosteroids.<br>17. Previous adverse side effects and/or contraindications to RASBs and SGLT2is.<br>18. Pregnancy and breastfeeding.<br>19. If women of childbearing potential (WOCBP): patients not available to use highly effective contraceptive measures during the study treatment period and up to one month after the last dose of study drugs. |
| **Study Products** | Methylprednisolone succinate and prednisone (Corticosteroids, CS)<br>Dapagliflozin (Sodium-glucose cotransporter 2 inhibitor, SGLT2i) |

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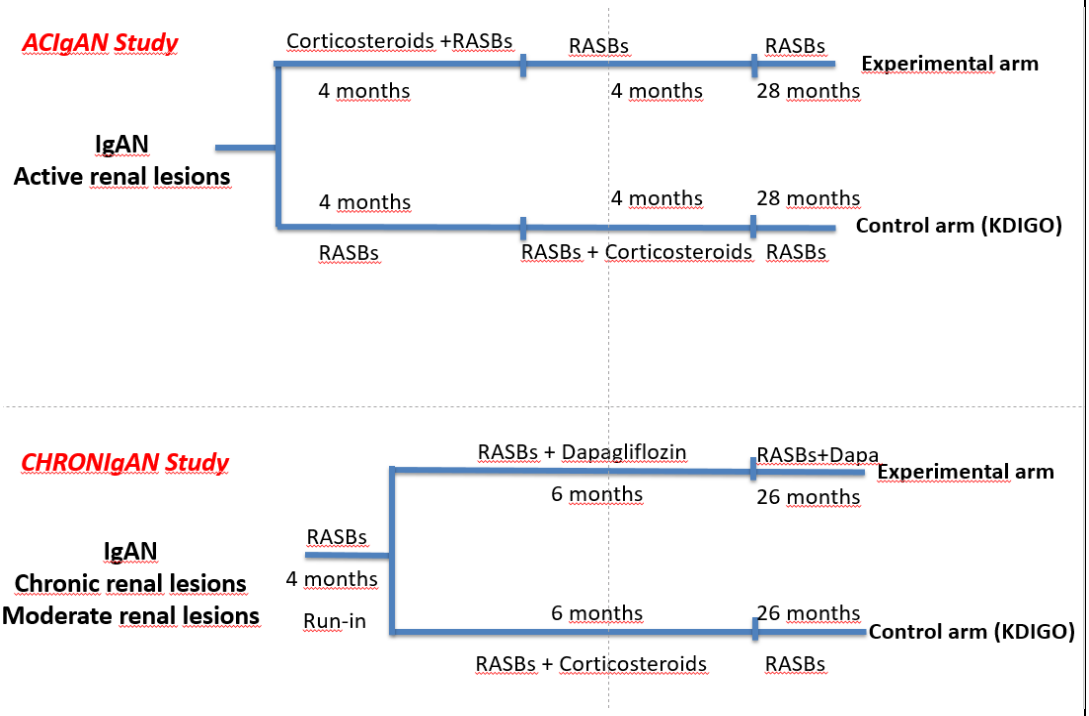
| **Treatments** |  <br>Patients will be randomized separately in each trial (allocation 1 to 1 ratio). Stratified randomization will be done by age and sex in renal centers.<br>**ACIgAN trial**<br>Experimental arm: Early corticosteroids therapy combined with RASBs followed by RASBs treatment.<br>Control arm (standard care): RASBs treatment followed by corticosteroids combined with RASBs and then only RASBs.<br>**CHRONIgAN trial**<br>Experimental arm: RASBs therapy followed by Dapagliflozin combined with RASBs.<br>Control arm (standard care): RASBs therapy followed by Corticosteroids combined with RASBs and then RASBs alone |
| **Sample Size and Statistics** | **ACIgAN**. Data from the literature have shown a difference in renal survival between corticosteroids and controls when the researchers assumed a difference of 50% in the primary end point (i.e., in the between-arms difference of delta 24 hour proteinuria from base-line to 6 months) as clinically relevant. Therefore, we assume that a mean delta proteinuria from base-line to 6 months in patients treated with RASBs alone (control arm) is 0.6±1.0 g/24 hours (±SD) versus a mean delta proteinuria 1.2±1.0 g/24 hours (±SD) from base-line to 6 months in patients treated with RASBs and corticosteroids (experimental arm). Based on these assumptions, we calculated a sample size of 132 patients (66 patients per group including a 10% dropout rate) for a power of 90%, a two-sided significance level of 0.05 and 3 years |

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| | of recruitment. If the number of the enrolled patients will not reach the expected sample size within the established timeframe, the recruitment period will be extended for two additional years.<br><br>**CHRONIgAN.** Data from the literature have shown a difference of renal survival between SGLT2is and controls. There are no data in the literature exploring the difference of renal survival or proteinuria between SGLT2is and steroids. Here, the researchers assumed an opportunistic sample of 300 patients for this first comparative study (150 patients per group, including a 10% dropout rate). |
| **Planned study timelines** | Enrollment period: 3 years<br>Treatment and Follow-up period: 3 years + 30 days |

## LIST OF ABBREVIATION

| | |
|---|---|
| ACEi | *Angiotensin Converting Enzyme inhibitors* |
| ACIgAN | *IgAN patients with ACtive renal lesions* |
| AE | *Adverse Event* |
| AKI | *Acute Kidney Injury* |
| ARB | *Angiotensin Receptor Blockers* |
| AZA | *Azathioprin* |
| BrdU | *Bromodeoxyuridine* |
| C | *Extracapillary proliferation, crescents* |
| CDSS | *Clinical Decision Support System* |
| CHRONIgAN | *IgAN patients with CHRONic renal lesions* |
| CI | *Confidence Interval* |
| CKD | *Chronic Kidney Disease* |
| CKD-EPI | *Chronic Kidney Disease Epidemiology Collaboration* |
| COVID-19 | *COronaVIrus Disease 2019* |
| CRO | *Contract Research Organization* |
| CS | *Corticosteroids* |
| CSR | *Clinical Study Report* |
| CXCL6 | *C-X-C Motif Chemokine Ligand 6* |
| CYP | *Cyclophosphamide* |
| DEFA4 | *Defensin Alpha 4* |
| DEGs | *Differentially Expressed Genes* |
| E | *Endocapillary hypercellularity* |
| ECG | *Electrocardiogram* |
| eCRF | *electronic Case Report Form* |
| eGFR | *Estimated Glomerular Filtration Rate* |
| ELISA | *Enzyme-Linked Immunosorbent Assay* |
| ESKD | *End-Stage Kidney Disease* |
| FACS | *Fluorescence-activated Cell Sorting* |
| FAR2 | *Fatty Acyl-CoA Reductase 2* |
| g/24 hours | *gram per 24 hours* |
| g/day | *gram per day* |
| g/g | *grams per grams* |
| GCP | *Guideline for Good Clinical Practice* |
| Gd-IgA1 | *Galactose-deficient Immunoglobulin A1* |
| GEE | *generalized estimating equations* |
| GFR | *Glomerular Filtration Rate* |
| HbA1c | *Hemoglobin A1c* |
| ICH | *International Conference on Harmonization* |
| IgA | *Immunoglobulin A* |
| IgAN | *Immunoglobulin A nephropathy* |
| IgG | *Immunoglobulin G* |
| IHC | *ImmunoHistoChemistry,* |

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| IP | *Investigational Product* |
| ITGAX | *Integrin Subunit Alpha X* |
| KDIGO | *Kidney Disease: Improving Global Outcomes* |
| LMM | *Linear Mixed Models* |
| LTB | *Lymphotoxin Beta* |
| M | *Mesangial hypercellularity* |
| $m^2$ | *square metre* |
| MD | *Medical Doctor* |
| mEq/L | *milliequivalents per Litre* |
| MESTC-score | *mesangial (M) and endocapillary (E) hypercellularity, segmental sclerosis (S), interstitial fibrosis/tubular atrophy (T), crescents (C) score* |
| mg/day | *milligram per day* |
| mg/dL | *milligram per deciliter* |
| mg/kg | *milligram per kilogram* |
| mg/kg/bw | *milligram per kilogram of body weight* |
| min. | *Minute* |
| ml/min | *milliliter /minute* |
| MMF | *Mycophenolate Mofetil* |
| mmHg | *millimeter of mercury.* |
| PBMC | *Peripheral Blood Mononuclear Cell* |
| PI3K/Akt | *Phosphatidylinositol 3-kinase/protein kinase B* |
| RASBs | *Renin-Angiotensin System Blockers* |
| RCTs | *Randomized Controlled Trials* |
| RNA | *RiboNucleic Acid* |
| RT-PCR | *Reverse Transcription -Polymerase Chain Reaction* |
| S | *Segmental glomerular sclerosis* |
| SAE | *Serious Adverse Event* |
| SD | *Standard Deviation* |
| SGLT2i | *Sodium-Glucose co-Transporter-2 Inhibitor* |
| T | *Tubular atrophy/interstitial fibrosis* |
| TA-P | *Time-Averaged Proteinuria* |
| TNFAIP6 | *Tumor Necrosis Factor Alpha Induced Protein 6* |
| TNFR | *Tumor Necrosis Factor Receptor* |
| WNT-B-catenin | *Wnt/β-catenin pathway* |
| WOCBP | *Women of ChildBearing Potential* |

# 1. INTRODUCTION

## 1.1. Background

What is known

Idiopathic Immunoglobulin A nephropathy (IgAN) is the most common biopsy-proven glomerulonephritis in the world. It is more prevalent in Asia than in Europe and the US (1). IgAN is estimated to affect more than 500.000 people in Europe and it is the leading nephritis in kidney-biopsied patients. Approximately 40% of IgAN patients reach end-stage kidney disease (ESKD) 20 years after their kidney biopsy (2,3). The high prevalence of ESKD shows that IgAN has a significant economic impact in the countries because renal replacement therapy is costly. Moreover, the disease's onset in the second and third decades of life represents a social challenge because patients are typically very active and highly productive in the workplace. This challenge is one more reason to move from a generalized therapy for all patients to personalized therapy.

The first edition of the KDIGO guidelines, published in 2012 (4), suggested different therapeutic approaches for IgAN patients based on the clinical setting. Three sets of patients were considered:

i) *Patients with low risk* manifest minor urinary findings and proteinuria < 0.5 g/day, normal estimated glomerular filtration rate (eGFR) and normal blood pressure; they need only annual or biannual checks without therapy. They represent only 5-10% of the IgAN patient population.

ii) *Patients with intermediate risk* have proteinuria >0.5 g/day with normal or reduced eGFR and normal or high blood pressure. They may benefit from continuous supportive therapy (renin-angiotensin system blockers; RASBs) when proteinuria lowers less than 1g/day, and eGFR remains stable. When proteinuria is > 1g/day and eGFR (normal at baseline) declines to >50 ml/min/ 1.73 $m^2$, after three months of continuous supportive therapy, patients may benefit from corticosteroids combined with supportive therapy for six months. When eGFR is less than 30 ml/min or between 30 and 50 ml/min/1.73 $m^2$, only supportive treatment is recommended with no immunosuppression, except for patients with rapidly progressive glomerulonephritis.

iii) *Patients at high risk* for acute or rapid loss of eGFR caused by rapidly progressive glomerulonephritis or nephrotic syndrome may benefit from corticosteroids combined with immunosuppressive agents and supportive therapy. These patients represent 2-5% of the IgAN population. In the presence of acute kidney injury (AKI) caused by red blood cells stacked in the tubules during the macrohematuria episode, only supportive therapy is suggested.

These indications have been confirmed in the recent edition of the KDIGO 2021 guidelines (5).

Unfortunately, the KDIGO guidelines could consider the presence of active and chronic renal lesions at the time of the kidney biopsy for therapy decision based on the lack of intervention evidence deriving from randomized trials. Nonetheless, such an approach would be supported, by the MEST-C score of the Oxford classification (6-8) validated in the VALIGA cohort (9). The MEST-C classification distinguishes active renal lesions (endocapillary proliferations (E) and extracapillary lesions C1-2) from chronic renal lesions (tubulo-interstitial damage T1,2).

In clinical settings, it is known that patients with active renal lesions are more prone to respond to immunosuppressive treatment than those with chronic lesions identified at kidney biopsy. In this

setting, approaching patients with chronic kidney lesions with immunosuppression may not be indicated, but this has not been formally explored in the setting of a randomized trial.

We have reviewed the effect of therapy (corticosteroids and/or immunosuppressive drugs) in biopsy-proven IgAN patients with active and chronic renal lesions based on cohort and registry data. Some of the most important publications have been listed in ***Table 1*** in which conclusions of the studies are reported. Retrospective and prospective clinical studies and meta-analyses show that corticosteroids and/or immunosuppressive therapy reduce the number of active renal lesions in repeated kidney biopsies (10-15). Moreover, there is an improvement in proteinuria and in kidney function (16-20). The same does not apply where chronic lesions are present.

**Table 1. Retrospective and Prospective clinical studies, Systematic Reviews and Meta-analyses of randomized clinical studies promoting the benefit of corticosteroids and/or immunosuppressive therapy in reversing the active renal lesions and improving the renal prognosis**

| Authors | Ref (no.) | Type of study | Conclusions |
|---|---|---|---|
| Shen XH et al. | 10 | Retrospective | IS reversed the active renal lesions (E1,C1,2), confirmed by repeat biopsy. |
| Moriyama et al | 11 | Retrospective | CS/IS improved the long-term renal prognosis of IgAN patients with E1, S1, C1 lesions. |
| Luo M et al. | 12 | Retrospective | Repeated kidney biopsies demonstrated that CS/IS reduce the number of crescents (C1,2). |
| Itami S. et al. | 13 | Retrospective | Presence of fibrinoid necrosis with crescents (C) or endocapillary (E) renal lesions is a pathological indicator of patients who may benefit from immunosuppressive therapy. |
| Tumulin JA et al. | 14 | Prospective | CS reduced proliferative lesions, proteinuria and stabilized renal function. |
| P. Yang et al. | 15 | Systematic Review | Patients with serious pathological changes (M1, S1 and T1,2) were more responsive to CS than patients with mild renal lesions (M0, S0, T0). E1 has better response to CS than T1,T2. |
| Tesar V et al. | 16 | Retrospective | CS reduced the risk of disease progression and proteinuria. |
| Guo Y et al. | 17 | Retrospective | Presence of fibrinoid necrosis with crescents (C) or endocapillary (E) renal lesions is a pathological indicator of patients who may benefit from immunosuppressive therapy. |
| Li Y et al. | 18 | Retrospective | Pulses of iv CS combined with low dose of prednisone is a new treatment option for high-risk IgAN patients. |
| P. Natale et al. | 19 | Systematic Review | CS therapy probably prevents decline in GFR or doubling of serum Creatinine (sCr) and proteinuria in adults and children with IgAN. |
| J. Tan et al. | 20 | Systematic Review | CS significantly delay the progression of renal damage with acceptable adverse events. They are recommended as the first line of immunosuppressive therapy. |

CS: Corticosteroids; IS: Immunosuppressors

Referenced studies (10-15) seem to fit some of the Bradford Hill criteria (21) for the ascertainment of causality, specifically i) there are several observational retrospective studies (10-12) and a prospective study (14) and a systematic review (15) exploring the association between the type of kidney biopsy lesions and response to immunosuppressive treatment; ii) there is consistency across studies in the findings that active lesions may respond to immunosuppression as opposed to chronic lesions, data which have been identified by researchers from different countries in the world; iii) there is a strong association between different type of renal lesions and response to immunosuppressive therapy. In view of well established pragmatic clinical experience and the number, consistency and strength of the findings of observational studies, there seems to be scientific equipoise for the design and conduct of a randomized trial of interventions for IgA nephropathy based on the findings of kidney biopsy.

What is not known

There are no randomized data on the different treatment of active and chronic renal lesions in IgAN patients. The last RCTs using steroids (STOP IgAN and TESTING) (22,23) did not evaluate the type of lesions in the kidney biopsy at the enrollment time. Patients with active and chronic renal lesions all received the same treatment approach, independent of kidney biopsy findings. In consideration of this, recently, the researchers of the TESTING study have programmed a post-hoc analysis to evaluate the effect of therapy on renal lesions (24).

In STOP IgAN and TESTING studies, patients were enrolled after 3-6 months of RASB therapy, as per the current standard care. A recent histology study (25) based on two consecutive protocol kidney biopsies showed the dynamic changes of active and chronic renal lesions during the clinical course of the disease. Therefore, after 3-6 months active renal lesions not only in IgAN but in other glomerulonephritis (ie lupus nephritis) become chronic and corticosteroids may therefore not exert any role. This point has been commented by Troyanov et al (26) in a recent paper published by Itami et al (13) although needs to be formally explored in the setting of an experimental randomized trial.

Why this study

We have designed the CLIgAN studies in which biopsy-proven IgAN patients with active and chronic renal lesions will receive different treatments in consideration of the above described points. Therefore, CLIgAN is the first project including randomized studies which will take into consideration the type of renal lesions and patients will be enrolled separately in two RCTs based on the presence of active and chronic biopsy lesions.

Recently, oral sodium-glucose cotransporter 2 inhibitors (SGLT2i) were administered in patients with diabetic nephropathy type 2 and in other participants with chronic kidney disease (CKD), some of them were affected by IgAN (27-31). The effect of SGLT2i was compared with a placebo. All patients received RASB therapy. Patients with IgAN who received SGLT2i had a significantly lower risk of a composite end point (sustained decline of eGFR of at least 50%, ESKD, or death from renal or cardiovascular causes), independent of the presence or absence of diabetes (30,31). All patients had received kidney biopsy many months or years before the enrollment.

In CLIgAN study the participants will receive for the first time Dapagliflozin after the kidney biopsy in presence of chronic or moderate renal lesions with high risk of CKD. This RCT will start on October 2022 when the drug will be available for all patients with CKD.

### 1.2. Rationale

In our CLIgAN study, we have planned to evaluate:

(i) The effect of early start corticosteroid therapy combined with RASBs (experimental arm) versus RASBs alone in IgAN patients with active renal lesions (ACIgAN) who will receive corticosteroids after 4 months of RASBs (standard care) in presence of persistent proteinuria ≥ 0.5 g/24 hours and eGFR ≥ 30 ml/min/1.73 $m^2$ (control arm).

(ii) The effect of SGLT2i (dapagliflozin) combined with RASBs (experimental arm) versus corticosteroids combined with RASBs (control arm) in IgAN patients with chronic or moderate renal lesions (CHRONIgAN) after a run-in phase of 4 months in which they will receive RASB therapy alone (standard care).

In both studies patients enrolled in the control arms will be treated according to the evidence-based recommendations (standard care) provided within the KDIGO guidelines.

In conclusion, we would like to evaluate the effect of early start corticosteroids in IgAN patients with active renal lesions (ACIgAN) and to spare corticosteroids therapy in IgAN with chronic or moderate renal lesions (CHRONIgAN) administering Dapagliflozin.

We would also provide earlier ESKD risk prediction by using our tool DialCheck (32).

### 1.3. Study products

#### Corticosteroids

##### Methylprednisolone (SOLU-MEDROL)

Methylprednisolone is a potent anti-inflammatory steroid. It has a greater anti-inflammatory potency than prednisolone and even less tendency than prednisolone to induce sodium and water retention. Its anti-inflammatory activity is at least five times that of hydrocortisone.

Methylprednisolone sodium succinate has the same metabolic and anti-inflammatory actions as methylprednisolone. When given parenterally and in equimolar quantities, the two compounds are equivalent in biologic activity. The relative potency of methylprednisolone sodium succinate and hydrocortisone sodium succinate, as indicated by depression of eosinophil count, following intravenous administration, is at least four to one.

Among other uses, it is indicated to induce remission of active renal lesions and proteinuria in IgAN patients (13,14,18) and in other renal diseases as idiopathic nephrotic syndrome (33,34) and lupus nephritis (35).

##### Prednisone

Prednisone, while exhibiting the typical glucocorticoid steroid profile, does differs from cortisone, from which it derives by dehydrogenation in position 1, 2 of the molecule, due to its ability to increase (on average 4 times compared to cortisone) all those pharmacological activities connected

with the antireactional properties of this class of compounds. Prednisone does not possess by itself a biological activity, but it becomes active in the organism as it undergoes, by hepatic reductases that reduce the ketone in position 11 to hydroxyl, a rapid conversion to prednisolone which represents the metabolite activated steroid. Under normal conditions this process takes place quickly and totally in the liver, so much so that prednisone and prednisolone can be found to be pharmacologically equivalent.

Studies conducted in different experimental models, in addition to demonstrating the remarkable anti-inflammatory activity of the cortisone, indicated that the prednisone is able to influence glyconeogenesis, to stimulate the deposition of hepatic glycogen, to determine an eosinopenizing effect, to prolong survival time in adrenalectomised animals, to protect rats from acute egg white poisoning, to inhibit weight growth and to antagonize the experimentally induced alterations in the endothelium of veins and capillaries. Finally, the experiences carried out on hydro-mineral excretion have shown that administering dose treatments of prednisone cause a beneficial increase in diuresis and elimination of sodium, without stimulating urinary excretion of potassium.

Prednisone has been administered in IgAN patients 10-12), in proliferative primary and secondary glomerulonephritis (36) to reduce the inflammatory process at glomerular level.

**Dapagliflozin (SGLT2i)**

Dapagliflozin is a highly potent (Ki: 0.55 nM), selective and reversible inhibitor of SGLT2.

Inhibition of SGLT2 by dapagliflozin reduces reabsorption of glucose from the glomerular filtrate in the proximal renal tubule with a concomitant reduction in sodium reabsorption leading to urinary excretion of glucose and osmotic diuresis. Dapagliflozin, therefore, increases the delivery of sodium to the distal tubule which increases tubuloglomerular feedback and reduces intraglomerular pressure. This combined with osmotic diuresis leads to a reduction in volume overload, reduced blood pressure, and lower preload and afterload, which may have beneficial effects on cardiac remodelling and preserve renal function. Other effects include an increase in haematocrit and reduction in body weight.

The cardiac and renal benefits of dapagliflozin are not solely dependent on the blood glucose-lowering effect and not limited to patients with diabetes as demonstrated in the DAPA-HF and DAPA-CKD studies (29,30).

Dapagliflozin improves both fasting and post-prandial plasma glucose levels by reducing renal glucose reabsorption leading to urinary glucose excretion. This glucose excretion (gluretic effect), observed after the first dose, is continuous over the 24-hour dosing interval and is sustained for the duration of treatment. Thus, Dapagliflozin is administered one time per day (29-31).

The amount of glucose removed by the kidney through this mechanism is dependent upon the blood glucose concentration and GFR. Thus, in subjects with normal blood glucose, dapagliflozin has a low propensity to cause hypoglycaemia. Dapagliflozin does not impair normal endogenous glucose production in response to hypoglycaemia. Dapagliflozin acts independently of insulin secretion and insulin action. Improvement in homeostasis model assessment for beta cell function (HOMA beta-cell) has been observed in clinical studies with Dapagliflozin.

The SGLT2 is selectively expressed in the kidney. Dapagliflozin does not inhibit other glucose transporters important for glucose transport into peripheral tissues and is > 1,400 times more

selective for SGLT2 versus SGLT1, the major transporter in the gut responsible for glucose absorption (37,38).

### 1.4. Renin-angiotensin system blockers (RASBs)

The role of the renin-angiotensin-aldosterone system in the pathophysiology of hypertension, and cardiovascular and kidney diseases is well known and the renin-angiotensin-aldosterone system is a major regulator of blood pressure through its effect on body fluids and electrolyte homeostasis. For 2 decades, RASBs have been the mainstay of treatment for CKD. Clinical trials have shown that prescription of monotherapy with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers reduces albuminuria and slows the progression of nephropathy in patients with diabetes. In clinical practice guidelines, RASBs are recommended as the antihypertensive drug of choice in patients with CKD with or without diabetes (39,40).

### 1.5. Overall risk and benefit statement

The major benefit for the IgAN patients enrolled in the present study will be a targeted therapy based on renal lesions described in the kidney biopsy report, given the existing equipoise for scientific studies that may assess the role of kidney biopsy in driving interventions, in light of significant empiric practice evidence and strong, consistent observational evidence supporting a potential causal link.

This may be an advantage for IgAN patients, in line with what is supported by the *MEST-C score* of the Oxford classification validated in the VALIGA study cohort (9).

To date, the current KDIGO guidelines do not consider the presence of active and chronic renal lesions at the time of the kidney biopsy for therapy decision, based on the lack of experimental trial data; this question seems highly relevant in view of pragmatic clinical practice knowledge and the results of cohort studies, their strength and consistency.

The potential risks and benefits of this trial are mainly related to study treatments and they are listed below; the actions/ways to mitigate the risks are specified, in brackets, in the section “Drug risks”.

#### Drug risks

Corticosteroids: hypertension (anti-hypertensive drugs), water retention (diuretics), hyperglycemia (antidiabetic drugs or insulin), muscle hypotrophy (daily exercise), hyperlipidemia (lipid-lowering agents), body weight increase (diet).

RASBs: allergy (anti-allergy drugs or low doses of corticosteroids), angioedema (corticosteroids), hypotension (dose reduction), hyperkalemia (diet or cation exchange resins), impairment of the renal function (dose reduction or temporary suspension), acute renal failure (drug suspension).

Dapagliflozin: allergy (anti-allergy drugs or low doses of corticosteroids), hyperlipidemia (lipid-lowering agents), hypoglycemia (drug suspension), impairment of the renal function (dose reduction or temporary suspension), acute renal failure (drug suspension).

#### Drug benefits

Proteinuria reduction, time-averaged proteinuria (TA-P) improvement, proteinuria slope reduction, eGFR slope reduction, delayed end-stage kidney disease.

## 2. OBJECTIVES

In our CLIgAN study we plan to evaluate:

### 2.1 Primary objectives

- To evaluate the therapeutic effect on proteinuria with early corticosteroid therapy, administered after the kidney biopsy in IgAN patients with active renal lesions (ACIgAN) versus standard care of RASBs alone followed by corticosteroids combined with RASBs;
- To evaluate the therapeutic effect of SGLT2i (Dapagliflozin) combined with RASBs on proteinuria versus corticosteroids combined with RASBs in patients with chronic or moderate renal lesions (CHRONIgAN); thus, to avoid the potential side effects of corticosteroid therapy.

### 2.2 Secondary objectives

- the effect of study treatments on the renal function and on the progression of the kidney damage in IgAN patients at 3 years
- after the prediction of ESKD through our IgAN CDSS tool (DialCheck) at the time of the kidney biopsy, to determine whether personalized therapy delays the impairment of the renal function.

## 3. TRIAL DESIGN

This project encompasses two prospective, multicentre, open-label randomized controlled clinical trials (RCTs) of IgAN patients (CLIgAN) based on the kidney biopsy report (***Figure 1***).

Stratified randomization will be done by age and sex in renal centers.

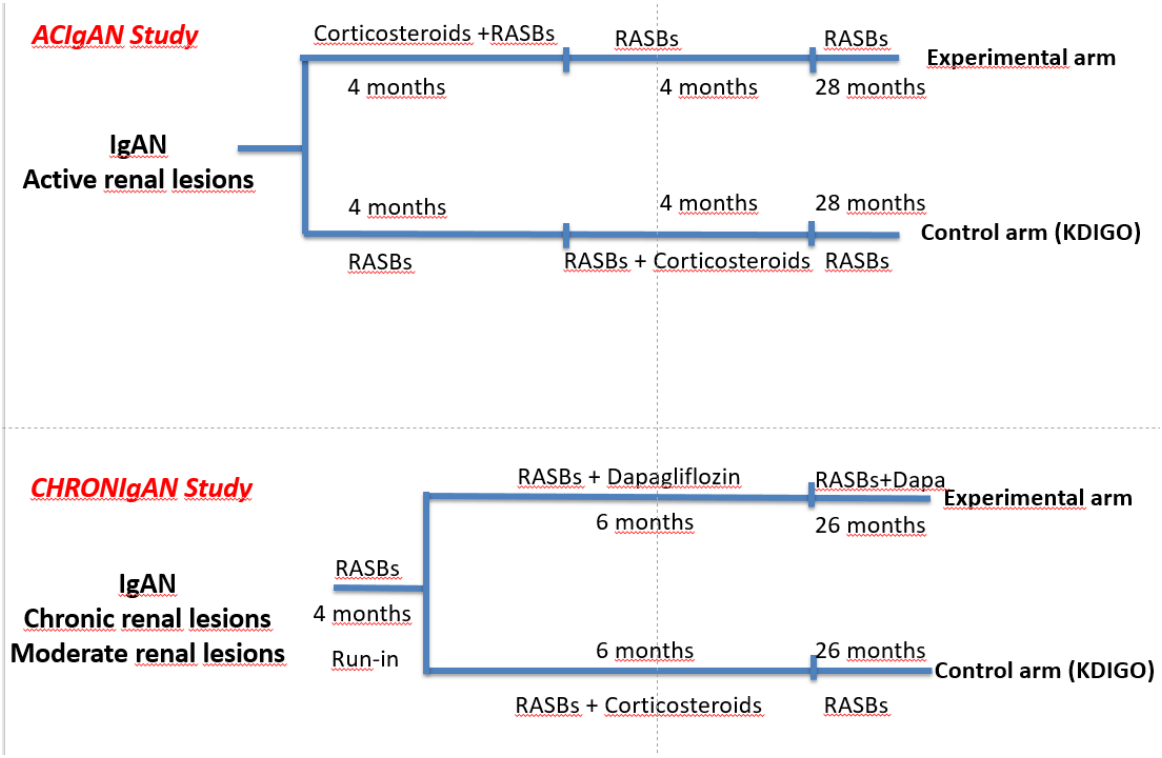


***Figure 1***

**ACIgAN TRIAL**

**Patients with active renal lesions** (E1 and/or C1) in the presence of GFR ≥ 30 ml/min/1.73 $m^2$ and proteinuria ≥ 0.5 g/24 hours will be randomized into two arms:

1) corticosteroids combined with RASBs for 4 months followed by RASBs alone (experimental arm);

2) RASBs alone for 4 months followed by the combination corticosteroids and RASBs for 4 additional months and then only RASBs (standard care) as suggested by KDIGO guidelines (5).

Participants assigned to the experimental arm will receive by 2 weeks from the kidney biopsy (pulse) methylprednisolone (SOLU-Medrol) 500-1000 mg in the first day and then 50% of dose in the next two days followed by oral prednisone (0.5 mg/kg/bw) on alternate days until the end of the month. This treatment will be repeated for three consecutive months. After three months, participants will gradually reduce oral prednisone, in the course of four weeks. Next, they will receive RASBs alone.

The daily dose of methylprednisolone will be individualized (15 mg/kg), based on the ideal body weight. The drug will be administered in a single dose intravenously for 30-60 min. To avoid obesity and diabetes corticosteroids will be administered only in the morning.

This is a parallel group, two arm, superiority trial with 1:1 allocation ratio.

As per standard care RASB therapy will be titrated to its maximum labelled or tolerated dose as anti-proteinuric effect in every patient.

**Rationale of the study design**

Corticosteroids at high dosage have been administered in RCTs of IgAN (18,41) independent of the type of renal lesions despite empiric evidence from clinical practice and strong and consistent evidence from observational studies that the lesions identified at kidney biopsy may play a role in the response to treatment. Our aim is to evaluate the benefit of early pulse therapy combined with oral prednisone only in IgAN patients with active renal lesions. In our protocol, we have excluded intensive immunosuppressive therapy (CS+CYP/AZA/MMF) because RCTs have demonstrated that the potential benefits-harms trade off is in favour of the harms (42).

To our knowledge, this is the first study in which corticosteroids are administered immediately after the kidney biopsy in IgAN patients with active renal lesions. Based on the existing evidence from randomized clinical trials, the KDIGO guidelines suggest in those cases: RASBs for 3-6 months and then corticosteroids in presence of persistent proteinuria and GFR >30 ml/min/1.73 $m^2$. This therapeutic approach, although supported by evidence, may be a challenge based on empiric clinical knowledge supported also by findings of cohort studies. This demonstrates that after 3-6 months active renal lesions become chronic and consequently no association may be found between exposure to corticosteroids and clinical response for IgAN patients. In our randomized clinical study we compare the standard approach proposed by KDIGO guidelines (RASBs for 3-6 months followed by corticosteroids) vs a novel approach consisting of early corticosteroid therapy combined with RASBs from the beginning.

**CHRONIgAN TRIAL**

**Patients with chronic** (T1,2) **or moderate renal lesions** (M0,1; S0,1, E0, C0, T0) will be enrolled in a two-arm trial with 1:1 allocation ratio to compare Dapagliflozin combined with RASBs (experimental arm) versus corticosteroids combined with RASBs (control arm) after 4 run-in months of RASBs treatment. The enrolled patients will have proteinuria ≥ 0.5 g/day and GFR ≥30ml/min/1.73 $m^2$.

Participants will receive study treatments by 4 weeks from the kidney biopsy.

Patients assigned to the experimental arm will receive RASBs therapy (4 run-in months) followed by Dapagliflozin (10 mg /day orally) combined with RASBs for a total of 32 consecutive months. Patients assigned to the control arm will receive RASBs therapy (4 run-in months) followed by oral corticosteroids therapy (prednisone) for six months (0.4 mg/kg body weight for 2 months and then gradual reduction of 5 mg/day for 4 months) and then RASBs alone for a total of 36 consecutive months.

This is a parallel group, two arm, superiority trial with 1:1 allocation ratio.

**Rationale of the study design**

We decided the combination Dapagliflozin and RASBs because recently, SGLT2is were administered in patients with type 2 diabetes and in other participants with CKD (some of them were affected by IgAN) (29-31). In these studies, the effect of SGLT2is was compared with a placebo.

In our RCTs oral RASBs will be titrated to their maximum anti-proteinuric effect. For example, Ramipril will be administered at a dose of 2.5 mg/day and then increased of 1.25 mg/day every 2 weeks to reduce proteinuria to 0.5 g/day or less and to achieve a systolic and diastolic pressure of <130/80 mmHg. We will monitor the outcomes to look at whether the experimental therapy may delay the time to reach the ESKD predicted by our IgAN tool DialCheck (32)

Patients will be converted to angiotensin receptor blockers (ARB) in presence of ACE-inhibitors (ACEi) intolerance for side effects. Ramipril will be administered two times a day (8 am and 8 pm) to control blood pressure. If necessary other anti-hypertensive drugs will be administered. Kalemia will be controlled so as not exceed 5.5 mEq/L.

In conclusion, we have designed two RCTs to study targeted therapy in biopsy-proven IgAN patients.

## 4. PARTICIPANTS, INTERVENTIONS AND OUTCOMES

### 4.1 Study setting

Patients aged 18 to 75 years, with biopsy-proven IgAN will be eligible to participate in the study.

### 4.2 Eligibility criteria

Inclusion and exclusion criteria are listed below. Patients will be enrolled in a time frame of three years and the duration of the follow-up will be three years.

### 4.2.1 Inclusion criteria

#### 4.2.1.1 ACIgAN study

1. Males and females aged 18 to 75 years.
2. Written informed consent form.
3. Biopsy-proven idiopathic IgAN with active renal lesions (E1 and/or C1) within 2 weeks.
4. eGFR ≥ 30 ml/min/1.73 $m^2$.
5. 24-hour proteinuria ≥ 0.5 g.
6. Patients on treatment or candidate for the treatment with RASBs (either an ACEi or ARB), as per clinical practice, according to the current KDIGO guidelines

#### 4.2.1.2 CHRONIgAN study

1. Males and females aged 18 to 75 years.
2. Written informed consent form.
3. Biopsy-proven idiopathic IgAN with chronic (T1,2) or moderate (M0,1, S0,1, E0, T0, C0) renal lesions at high or very high CKD risk within 4 weeks.
4. eGFR ≥ 30 ml/min/1.73 $m^2$.
5. 24-hour proteinuria ≥ 0.5 g.
6. Patients on treatment or candidate for the treatment with RASBs (either an ACEi or ARB), as per clinical practice, according to the current KDIGO guidelines.

### 4.2.2 Exclusion criteria

1. Non biopsy-proven IgAN
2. IgAN patients with minimal change disease at kidney biopsy and nephrotic syndrome.
3. IgAN patients with macrohematuria and acute renal failure.
4. IgAN patients with rapidly progressive glomerulonephritis (extracapillary lesions in more than 25 % of glomeruli in the kidney biopsy).
5. Patients with secondary IgAN (lupus nephritis, Schoenlein-Henoch purpura, liver cirrhosis).
6. Patients with superimposed IgAN in a kidney transplant.
7. Patients with other types of glomerular diseases
8. Patients with solitary kidney
9. Patients with end-stage kidney disease
10. Bleeding disorders not responsive to treatment
11. Patients with myocardial infarction or cerebrovascular stroke in the previous six months.
12. Severe liver diseases, infections, malignancies.
13. Uncontrolled diabetes (glycemia > 200 mg/dL and HbA1c > 7.5%).

14. Aseptic necrosis of any bone.
15. Any prior immunosuppressive therapy.
16. Other morbidities that can be exacerbated by corticosteroids.
17. Previous adverse side effects and/or contraindications to RASBs and SGLT2is.
18. Pregnancy and breastfeeding.
19. If women of childbearing potential (WOCBP): patients not available to use highly effective contraceptive measures during the study treatment period and up to one month after the last dose of study drugs.

## 4.3 Interventions

### 4.3.1 Study treatments (dosage and rationale)

Study drugs (already been placed on the market as authorized medicinal products) will be not supplied by the study Sponsor and will be administered according their labelling and no additional labelling will be required to ensure subject safety and the reliability and robustness of data generated in this clinical trial.

For all information about study drugs, please refer to their respective Summary of Product Characteristics (SmPCs).

**RASBs**

Patients will receive treatment with renin-angiotensin system blockers, RASBs (either angiotensin-converting enzyme inhibitor, ACEi, or angiotensin II receptor blocker, ARB) as per clinical practice, according to the current KDIGO guidelines.

For example, Ramipril will be administered at dosage of 5 mg x 2 times per day (8.00-20.00) in patients with blood hypertension. Then, it will be increased of 2.5 mg x 2 per day every three days until the reduction of hypertension and proteinuria.

In patients with normal arterial blood pressure Ramipril will be administered at dosage of 2.5 mg x 2 times a day

In patients no tolerant to Ramipril, a sartan will be administered, i.e. Losartan 12.5 mg x 2 times a day. A gradual increase of 6.25 mg (two times a day) will be done to reduce proteinuria.

Rationale: RASBs will be administered to reduce blood pressure at glomerular level and proteinuria (39,40 ).

**Dapagliflozin**

The drug will be administered at dosage of 10 mg per day as reported in other randomized clinical trials in which Dapagliflozin has been administered in combination with RASBs (29,31).

Rationale: To reduce blood pressure at glomerular level and proteinuria.

**Corticosteroids**

ACIgAN trial

Methylprednisolone will be administered at dosage of 15 mg/kg body weight until the dosage of 1 gr in the first day and 50% reduced dose in the other two days and then Prednisolone at dosage of 0.5 mg/kg body weight at alternate days will be administered until the end of the month. This treatment will be repeated for 3 consecutive months. After 3 months, prednisone will be gradually reduced of 25% of the dosage every week until the end of treatment (4 weeks).

Rationale: To care the active renal lesions represented by endocapillary and extracapillary glomerular lesions. This treatment has been used in other clinical trials of IgAN (12,18,43).

CHRONIgAN trial

Patients will receive oral corticosteroids therapy for six months (prednisone 0.4 mg/kg body weight for 2 months and then gradual reduction of 5 mg/day for 4 months) combined with RASBs and, then, RASBs alone for a total of 36 consecutive months.

Rationale: To care the moderate and chronic renal lesions in non-responders to RASBs alone as suggested by the KDIGO guidelines (4,5).

### 4.3.2 Lifestyle modifications and other therapy

**Concomitant therapy**

Eradication of infectious foci, or concurrent antibiotic therapy to prevent infections or to avoid the transformation of a trivial infection into a severe complication will be considered. In presence of reduced kidney function (eGFR < 30 ml/min/1.73 $m^2$) patients will receive reduced doses of nephrotoxic antibiotics.

Non-steroidal anti-inflammatory drugs and other nephrotoxic drugs will be avoided.

Due to the short term of corticosteroid therapy, IgAN patients will not receive supplementary therapy for osteoporosis. However, the physician will decide, in every patient, the supplementary therapy.

Inactivated pneumococcal and influence vaccines will be administered. Cotrimoxazole will be administered as Pneumocystis jirovecii prophylaxis.

COVID-19 Vaccine will be administered also in IgAN patients who will be enrolled in the arm of corticosteroids.

Patients treated with corticosteroids will receive ciprofloxacin before and during the two weeks following the meningococcal vaccination.

In presence of hypertension, anti-hypertensive drugs will be mainly replaced by RASBs to reduce hypertension and proteinuria.

Diuretics will be administered. Thiazides will be replaced by loop diuretics in presence of impairment of the renal function less than 30 ml/min/1.73 $m^2$.

Cardioprotective drugs will be administered in presence of cardiac insufficiency.

Statins will be prescribed in presence of hypercholesterolemia (> 200 mg/dL). Allopurinol will be administered in presence of hyperuricemia. It will be reduced if patients will have an impairment of the renal function less than 30 ml/min/1.73 $m^2$.

Cimetidine will not be prescribed because it interferes with serum creatinine measurements. Other gastroprotective drugs will be administered.

In presence of persistent or permanent eGFR <15 ml/min/1.73 $m^2$ patients will receive periodic hemodialysis or peritoneal dialysis.

Any other immunosuppressive therapies e.g. mycophenolate mofetil, cyclophosphamide or azathioprine will be not permitted in this study, unless there will be other definite indications for using these drugs.

Rifampin will be also prohibited from this study as it interacts with methylprednisolone and makes the study drug less effective.

**Life style modifications**

Regular daily exercise to prevent obesity and cardiovascular disease will be prescribed. Physical activity will be observed for 30 min. every day, primarily in the morning.

Patients with CKD stage 1-3 will observe the Mediterranean diet combined with reduced intake of animal proteins (0.8 g/kg bw/day). Salt (sodium chloride) intake will be limited to 5.0 g/day. Dietary compliance will be assessed by measuring daily urinary sodium and urea excretion.

A smoking intervention program will be done to stop smoking.

**Rescue therapy**

In patients with active renal lesions the rescue therapy will be represented by RASBs alone because, after long-term treatment from the kidney biopsy, they can be affected by only chronic renal lesions.

Finally, renal replacement therapy (dialysis or kidney transplantation).

In patients with moderate / chronic renal lesions the rescue therapy will be represented by RASBs combined with corticosteroids (oral prednisone).

### 4.3.3 Women of childbearing potential

A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

For WOCBP, a negative highly sensitive urine or serum pregnancy test will be done to include the patient in the trial.

Additional pregnancy testing during the trial will be performed if clinically indicated.
In each case of delayed menstrual period (over one month between menstruations) confirmation of absence of pregnancy will be ascertained. This protocol will be also applied to WOCBP with infrequent or irregular menstrual cycles.

The inclusion of WOCBP will require use of highly effective contraceptive measures during the study treatment period and up to one month after the last dose of study drugs.

According to the current version of "Recommendations related to contraception and pregnancy testing in clinical trials" of the *Clinical Trials Facilitation and Coordination Group*, methods that can achieve a failure rate of less than 1% per year when used consistently and correctly will be considered as highly effective birth control methods. Such methods will include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - oral
  - intravaginal
  - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable
  - implantable
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomized partner
- sexual abstinence.

### 4.4 Outcome measures

The primary endpoint is the 24 h-proteinuria reduction between arms as suggested by Inker LA et al (44). It will be assessed after 4 and 8 months in the ACIgAN study and after 10 months in the CHRONIgAN trial.

The secondary end point is the GFR slope calculated as the mean of individual slope obtained from individual linear regression of eGFR overtime (3 years). Based on the International Consensus definition of clinical trial outcomes for kidney failure (23) we have also considered other secondary end points listed below.

Primary and secondary end points that will be evaluated in the two RCTs are listed below:

<u>Primary endpoint:</u>

- Between-arms difference in proteinuria reduction within 4 and 8 months in ACIgAN and within 10 months in CHRONIgAN study.

<u>Secondary endpoints:</u>

- eGFR slope calculated as mean of individual slope obtained from individual linear regression of eGFR overtime (3 years);
- eGFR decline > 40 % from the baseline value;
- composite end point: GFR decline > 40%, ESKD (defined as long-term GFR ≤ 15 ml/min/1.73m$^2$ for more than three months or need for maintenance dialysis or kidney transplantation) or death due to kidney disease;
- absolute difference between last GFR value and baseline GFR;
- stable renal function defined as a decline in GFR ≤ 5 ml/min/1.73m$^2$ at the end of three years follow- up;
- mean annual change in the slope of the reciprocal of serum creatinine concentration;
- time-averaged proteinuria (TA-P) calculated as the weighted mean of all post- randomization measurement, with weights representing the time elapsed since the previous measurement;
- proteinuria slope, calculated as a mean of individual slope, obtained from individual linear regression of daily proteinuria overtime (3 years);
- complete remission of proteinuria defined as achievement of urinary protein level ≤ 0.2 g/day or a urinary protein-to-creatinine ratio ≤ 0.2 g/g;
- partial remission of proteinuria defined as achievement of a urinary protein reduction ≥ 50% or greater compared with baseline value.

The end points will be evaluated monthly in IgAN patients with active renal lesions in the first eight months after kidney biopsy and then every three months. In IgAN patients with chronic or moderate renal lesions the end points will be evaluated every three months in the first two years after the enrollment and then every six months.

### 4.4.1 Risk prediction

Risk prediction of ESKD in IgAN patients will be based on histology (MEST-C classification), eGFR, proteinuria, presence of hypertension at time of the kidney biopsy. We will use our IgAN CDSS tool (DialCheck).

By using a new designed tool, risk prediction will be done at different time of the clinical course of the disease: i) at the time of clinical and histological diagnosis; ii) after 4 and 8 months from the therapy in patients with active renal lesions; iv) after 10 months from the therapy in patients with chronic or moderate renal lesions. Then, every year during the follow-up.

## 4.5 Participant timeline

Total Study duration: approximately 6 years:

- Enrollment period: 3 years
- Treatment and Follow-up period: 3 years + 30 days.

***Figure 2A*** and ***2B*** illustrate the time schedule of enrollment, interventions, assessments and visits for participants.

**Figure 2A. SPIRIT 2013 STATEMENT OF THE ACIgAN STUDY: Time schedule of the enrolment, interventions, assessments and visits of the participants.**

| | **STUDY PERIOD** | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Enrol-ment** | **Alloca-tion** | **Post-allocation** | | | | | | | | | | | | | | **End of Treatment** | **End of Study** |
| **Time point** | - $t_1$ | $t_0$ | $t_1$ | $t_2$ | $t_4$ | $t_6$ | $t_8$ | $t_{12}$ | $t_{15}$ | $t_{18}$ | $t_{21}$ | $t_{24}$ | $t_{27}$ | $t_{30}$ | $t_{33}$ | $t_{36}$ | $t_{36}$ | 30 days after last treatment |
| **Enrolment:** | | | | | | | | | | | | | | | | | | |
| Eligibility screen | x | | | | | | | | | | | | | | | | | |
| Informed consent | x | | | | | | | | | | | | | | | | | |
| Kidney biopsy | x | | | | | | | | | | | | | | | | | |
| Laboratory data | x | | | | | | | | | | | | | | | | | |
| Allocation | | x | | | | | | | | | | | | | | | | |
| **Interventions:** | | | | | | | | | | | | | | | | | | |
| (A) Cs+RASBs (4 months) → RASBs | | | ● | — | — | — | — | — | — | — | — | — | — | — | — | ● | | |
| (B) RASBs (4 months) →Cs+RASBs (4 months)→RASBs | | | ● | — | — | — | — | — | — | — | — | — | — | — | — | ● | | |
| **Assessments:** | | | | | | | | | | | | | | | | | | |
| Serum creatinine | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | | |
| eGFR | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | | |
| Daily proteinuria | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | | |
| Adverse events | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | | x |
| Concomitant medications | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | | x |
| <u>Outcome variables:</u> | | | | | | | | | | | | | | | | | | |
| Proteinuria reduction (primary end point) | | | | | | x | x | x | x | x | x | x | x | x | x | x | x | |
| eGFR slope reduction (secondary end point) | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | |
| **Biological samples collection** | | x | x | x | x | x | x | x | | | | x | | | | x | | |

**Figure 2B. SPIRIT 2013 STATEMENT OF THE CHRONIgAN STUDY: Time Schedule of the enrolment interventions, assessments and visits of the participants.**

| | **STUDY PERIOD** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Enrol-ment** | **Alloca-tion** | **Post-allocation** | | | | | | | | | | | | **End of Treatment** | **End of Study** |
| **Time point** | - $t_1$ | $t_0$ | $t_1$ | $t_4$ | $t_8$ | $t_{10}$ | $t_{12}$ | $t_{14}$ | $t_{18}$ | $t_{24}$ | $t_{27}$ | $t_{30}$ | $t_{33}$ | $t_{36}$ | $t_{36}$ | 30 days after last treatment |
| **Enrolment:** | | | | | | | | | | | | | | | | |
| Eligibility screen | x | | | | | | | | | | | | | | | |
| Informed consent | x | | | | | | | | | | | | | | | |
| Kidney biopsy | x | | | | | | | | | | | | | | | |
| Laboratory data | x | | | | | | | | | | | | | | | |
| Allocation | | x | | | | | | | | | | | | | | |
| **Interventions:** | | | | | | | | | | | | | | | | |
| (A) RASBs (4 months)→ Dapagliflozin +RASBs | | | • | — | — | — | — | — | — | — | — | — | — | • | | |
| (B) RASBs (4 months)→ Cs+RASBs(6 months)→RASBs | | | • | — | — | — | — | — | — | — | — | — | — | • | | |
| **Assessments:** | | | | | | | | | | | | | | | | |
| Serum creatinine | x | | x | x | x | x | x | x | x | x | x | x | x | x | | |
| eGFR | x | | x | x | x | x | x | x | x | x | x | x | x | x | | |
| Daily proteinuria | x | | x | x | x | x | x | x | x | x | x | x | x | x | | |
| Adverse events | x | | x | x | x | x | x | x | x | x | x | x | x | x | | x |
| Concomitant medications | x | | x | x | x | x | x | x | x | x | x | x | x | x | | x |
| <u>Outcome variables:</u> | | | | | | | | | | | | | | | | |
| Proteinuria reduction (primary end point) | | | | | | x | x | x | x | x | x | x | x | x | x | |
| eGFR slope reduction (secondary end point) | | | x | x | x | x | x | x | x | x | x | x | x | x | x | |
| **Biological samples collection** | | x | x | x | x | x | | x | | x | | | | x | | |

### 4.6 Collection of biological samples

Blood (8 ml) and morning urine (30 ml) samples will be collected from IgAN patients with acute renal lesions enrolled in the ACIgAN clinical study and from IgAN patients with chronic and moderate renal lesions enrolled in the CHRONIgAN trial. Samples will be collected at the time points reported in the *Figure 2A* and *Figure 2B.* Blood and urine samples will be used for cystatin C measurement. The measurement of this variable will permit to obtain the eGFR creatinine/cystatin C, used to better evaluate the renal damage progression.

All samples will be stored in freezer at -20° or -80°C in the participant renal centers. Every six months, or when possible, the collected biological samples will be shipped to the Schena Foundation for the molecular study. Shipping fees will be supported by the Foundation.

For the collection of biological samples for the ancillary study "Cutting-edge bio-molecular analysis for precision therapy in IgAN (BIO-D), please refer section *No. 9. ANCILLARY PROJECT*.

### 4.7 End of Study and Patient withdraw

The end of the study is defined as the last visit of the last enrolled subject.

A visit of end of study will be performed 30 days after last treatment to collect data about any adverse events and other administered therapies.

The withdraw of enrolled patients from study treatment and/or from the whole study will be done in the following conditions:

- withdraw of the informed consent
- pregnancy
- superimposed malignant diseases
- kidney failure (end-stage kidney disease)
- dialysis
- renal transplant
- non-compliance with study procedures
- Investigator judgment, for any ethical, medical, or scientific reasons
- Death.

The patient may withdraw from the treatment/trial, at any time, without penalty or loss of benefits to which he/she is otherwise entitled.

## 5. ASSESSMENT OF SAFETY

The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section.

### 5.1 Safety parameters

#### 5.1.1 Definition of adverse events

The International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP) E6(R2) defines an adverse event (AE) as:

Any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE includes but is not limited to any clinically significant worsening of a patient's pre-existing condition. An abnormal laboratory finding (including ECG finding) that requires an action or intervention by the investigator, or a finding judged by the investigator to represent a change beyond the range of normal physiologic fluctuation, should be reported as an AE.

AEs s may be treatment emergent (i.e., occurring after initial receipt of investigational product) or non-treatment emergent. A non-treatment-emergent AE is any new sign or symptom, disease, or other untoward medical event that begins after written informed consent has been obtained but before the patient has received investigational product.

Elective treatment or surgery or preplanned treatment or surgery (that was scheduled prior to the patient being enrolled into the study) for a documented pre-existing condition, that did not worsen from baseline, is not considered an AE (serious or non-serious). An untoward medical event occurring during the prescheduled elective procedure or routinely scheduled treatment should be recorded as an AE or serious (S)AE.

The term AE is used to include both serious and non-serious AEs.

#### 5.1.2 Definition of serious adverse events

A serious adverse event (SAE) is an AE occurring during any study phase (i.e., screening, run-in, treatment, wash-out, follow-up), at any dose of the study drugs that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect in offspring of the patient
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above.
    - o Medical or scientific judgment should be exercised in deciding whether expedited reporting is appropriate in this situation. Examples of medically important events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalizations; or development of drug dependency or drug abuse.

The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to the Sponsor.

#### 5.1.3 Definition of adverse drug reactions

An Adverse Drug Reaction (ADR) is a response to a medicinal product which is noxious and unintended. This definition includes noxious and unintended effects resulting not only from the authorised use of a medicinal product at normal doses, but also from medication errors and uses outside the terms of the marketing authorisation, including the overdose, misuse and abuse of the medicinal product.

The definition implies a reasonable possibility of a causal relationship between the event and the IMP. This means that there are facts (evidence) or arguments to suggest a causal relationship.

A "serious" adverse reaction is an adverse reaction considered 'serious' according to the criterion of 'seriousness' reported in the previous section "Definition of serious adverse events".

An "unexpected adverse reaction" is defined as 'an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unauthorised investigational product or summary of product characteristics for anauthorised product)'.

### 5.2 Assessment of safety parameters

#### 5.2.1 Assessment of severity

Assessment of severity is one of the responsibilities of the investigator in the evaluation of AEs and SAEs.

The determination of severity should be made by the investigator based upon medical judgment and the severity categories of Grade 1 to 5 as defined below.

Grade 1 (mild): An event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Grade 2 (moderate): An event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the patient.

Grade 3 (severe): An event that requires intensive therapeutic intervention. The event interrupts usual activities of daily living, or significantly affects the clinical status of the patient.

Grade 4 (life-threatening): An event, and/or its immediate sequelae, that is associated with an imminent risk of death or with physical or mental disabilities that affect or limit the ability of the patient to perform activities of daily living (eating, ambulation, toileting, etc).

Grade 5 (fatal): Death (loss of life) as a result of an event.

It is important to distinguish between serious criteria and severity of an AE. Severity is a measure of intensity whereas seriousness is defined by the criteria in the previous section.

A Grade 3 AE need not necessarily be considered an SAE. For example, a Grade 3 headache that persists for several hours may not meet the regulatory definition of an SAE and would be considered a non-serious event, whereas a Grade 2 seizure resulting in a hospital admission would be considered an SAE.

#### 5.2.2 Assessment of relationship

Assessment of relationship to either investigational product or study procedures will be made according to investigator's best judgement.

### 5.3 Recording of AEs and SAEs

AEs and SAEs will be collected from the time of the patient signing the informed consent form until 30 days after last treatment. If an event that starts post the defined safety follow up period noted above is considered to be due to a late onset toxicity to study drug, then it should be reported as an AE or SAE as applicable.

During the course of the study, all AEs and SAEs should be proactively followed up for each patient for as long as the event is ongoing. Every effort should be made to obtain a resolution for all events, even if the events continue after the patient has discontinued study drug or the study has completed.

Any AEs that are unresolved at the patient's last visit in the study are followed up by the Investigator for as long as medically indicated, but without further recording in the eCRF. Sponsor retains the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

The following variables will be collected for each AE:

- AE (verbatim)
- The date when the AE started and stopped
- The maximum grade reported
- Changes in grade
- Whether the AE is serious or not
- Investigator causality rating against the investigated products (IPs) (yes or no)
- Action taken with regard to IPs
- Administration of treatment for the AE
- Outcome

In addition, the following variables will be collected for SAEs:

- Date of the AE met criteria for SAE
- Date of the Investigator became aware of the SAE
- Seriousness criteria fulfilled

- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death
- Whether an autopsy was performed
- Causality assessment in relation to study procedure(s)
- Causality assessment in relation to other medication, as explained in Section 9.3.2
- Description of the SAE.

### 5.3.1 Study recording period and follow-up for AEs and SAEs

AEs and SAEs will be recorded from time of signature of informed consent, throughout the treatment period and 30 days after last treatment.

During the course of the study all AEs and SAEs should be proactively followed up for each patient. Every effort should be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion.

The investigator is responsible for following all SAEs until resolution, until the patient returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation.

### 5.3.2 Causality collection

The Investigator will assess causal relationship between the IPs and each AE and answer “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?”

For SAEs causal relationship will also be assessed for other medications and study procedures. For SAEs that could be associated with any study procedure, the causal relationship is implied as “yes.”

### 5.3.3 Relationship to protocol procedures

The Investigator is also required to provide an assessment of the relationship of SAEs to protocol procedures on the SAE report form. This includes both non-treatment–emergent (i.e., SAEs that occur prior to the administration of IP) and treatment-emergent SAEs. A protocol-related SAE will be used as a result of a procedure or intervention required during the study (e.g., blood collection). The following guidelines will be used by the investigators to assess the relationship of SAEs to the protocol:

- Protocol related: The event occurred due to a procedure or intervention that has been described in the protocol for which there is no alternative etiology present in the patient’s medical record.

• Not protocol related: The event is related to an etiology other than the procedure or intervention that is described in the protocol. The alternative etiology must be documented in the study patient's medical record.

#### 5.3.4 Adverse events based on signs and symptoms

All AEs spontaneously reported by the patient or reported in response to the open question from the study personnel: "Have you had any health problems since the previous visit/you were last asked?" or revealed by observation will be collected and recorded in the eCRF. When collecting AEs, the recording of diagnoses will be preferred, when possible, to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

#### 5.3.5 Adverse events based on examinations and tests

The results from protocol-mandated laboratory tests and vital signs measurements will be summarized in the Clinical Study Report (CSR). Deterioration as compared to baseline in protocol-mandated laboratory values and vital signs will be reported as AEs if they fulfill any of the SAE criteria or are the reason for discontinuation of treatment with the IPs.

If deterioration in a laboratory value or vital sign will be associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result or vital sign will be considered as additional information. Whenever possible, the reporting Investigator will use the clinical rather than the laboratory term (e.g., anemia versus low hemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters will be reported as AEs.

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

#### 5.3.6 Deaths

All deaths that will occur during the study treatment period, or within the protocol-defined follow-up period after the administration of the last dose of study drug, will be reported to the Study Monitor/Physician as a SAE within 24 hours. It will be also documented in the eCRF.

Sponsor has the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

#### 5.3.7 Follow-up of unresolved adverse events

Any AEs that are unresolved at the patient's last visit in the study will be followed up by the investigator for as long as medically indicated, but without further recording in the eCRF. After 30 days from last treatment, only patients with ongoing investigational product-related SAEs will continue to be followed for safety.

Sponsor has the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

#### 5.3.8 Reporting of serious adverse events

All SAEs will be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). The reporting period for SAEs will be the period immediately following the time that written informed consent will be obtained through 30 days after last treatment or until the initiation of alternative therapy. The investigator and/or Sponsor will be responsible for informing the Ethics Committee and/or the Regulatory Authority of the SAE as per local requirements.

The sponsor will also indicate the causality of events in relation to all study medications, as determined by the principal investigator. A SAE report will be sent via email to the safety desk within 24 hours.

#### 5.3.9 Reporting of Suspected Unexpected Serious Adverse Reactions

Fatal or life-threatening suspected unexpected serious adverse reactions (SUSARs) will be reported by the Sponsor to the concerned regulatory Authority(ies) and to the Ethics Committee, within 7 calendar days after knowledge according to applicable regulations. The relevant follow-up information will be subsequently communicated within an additional 8 days.

All other SUSARs will be reported by the Sponsor to the concerned regulatory Authority(ies) and to the Ethics Committee with expedited reporting, within a maximum of 15 calendar days of first knowledge. The relevant follow-up information will be subsequently communicated as soon as possible.

The Sponsor will also inform all Investigators.

### 5.4 Pregnancy

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on investigational products, or within one month of the subject's last study treatment, will be considered immediately reportable events. Treatment will be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test will be reported to the sponsor immediately using pregnancy the Reporting Form. The Investigator will follow the female subject until completion of the pregnancy, and will notify to the StudySponsor immediately about the outcome of the pregnancy (either normal or abnormal outcome). If the outcome of the pregnancy will be abnormal (e.g., spontaneous or therapeutic abortion), the Investigator will report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it will be reported as an SAE within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

## 6. SAMPLE SIZE AND STATISTICAL METHODS

**ACIgAN**. Data from the literature (22,23) have shown a difference in renal survival between corticosteroids and controls when the researchers assumed a difference of 50% in the primary end point (i.e. in the between-arms difference of delta 24 hour proteinuria from baseline to 6 months) as clinically relevant. Therefore, we assume that a mean delta proteinuria from baseline to 6 months in patients treated with RASBs alone (control arm) is 0.6±1.0 g/24 hours (±SD) versus a mean delta proteinuria that is 1.2±1.0 g/24 hours (±SD) from baseline to 6 months in patients treated with RASBs and corticosteroids (experimental arm). Based on these assumptions, we calculated a sample size of

132 patients (66 patients per group including a 10% dropout rate) for a power of 90%, a two-sided significance level of 0.05 and 3 years of recruitment. If the number of the enrolled patients will be insufficient, the recruitment period will be extended for two years.

**CHRONIgAN.** Data from the literature (29-31) have shown a difference of renal survival between SGLT2is and controls. There are no data in the literature exploring the difference of renal survival or proteinuria between SGLT2is and steroids. Here, the researchers assumed an opportunistic sample of 300 patients for this first comparative study (150 patients per group, including a 10% dropout rate).

### 6.1 Recruitment

Rationale: In some RCTs (22,23) IgAN patients received three - six months of RASBs treatment before their enrollment for corticosteroid therapy. In our opinion this approach is not correct because the active renal lesions evolve in the chronic stage and are no-responsive to corticosteroid therapy. Therefore, the aim of our RCT is early treatment of the active renal lesions because they are responsible for altered GFR and proteinuria.

Recruitment: IgAN patients will be enrolled within 2-4 weeks from the kidney biopsy in renal centers. The kidney biopsy will be scored according to the Oxford classification (6-8) because it is a simple method for predicting renal outcomes and for scoring active and chronic renal lesions (46). Therefore, the kidney biopsy will be the principal key-note, not only for diagnosis but also for personalized therapy, because active renal lesions (E1,C1) will be treated with immediate corticosteroid therapy before lesions become chronic.

The kidney biopsy will be analyzed using digital histopathologic analysis coupled with machine-learning tools. At least eight glomeruli will be available for a correct diagnosis. Immunofluorescence technique will be used to determine the relevance of mesangial IgA deposits in the glomeruli. Renal tissue sections will be stained with hematoxylin and eosin, periodic acid-Schiff and methenamine silver. The last edition of the Oxford classification (MESTC) (8) will be used for the scoring of the renal lesions. Active renal lesions are endocapillary (E) and extracapillary (C) proliferative lesions while T lesions (T1,2) are expression of chronic renal lesions and severe outcome (46-49). Other histological lesions will be evaluated as fibrinoid necrosis, global glomerulosclerosis and arteriolosclerosis.

Three independent renal pathologists, blinded to the study results, will score the lesions in the kidney biopsy using the Aperio System. The histology report will be necessary for the enrollment of patients. No larger RCT has confirmed that the disease improves when clinical decisions are made in a short time in the presence of the MESTC score.

-Serum creatinine. Creatinine will be measured using enzymatic methods calibrated to the National Institute of Standards and Technology Liquid Chromatography Isotope Dilution Mass spectrometry method. Estimated (e) GFR creatinine will be estimated by the CKD-EPI formula (50). Moreover, we will calculate the slope of acute, chronic and total eGFR (51). Patients, categorized in CKD stage 1 to 3, will be included in the clinical study.

-Proteinuria. Patients will collect 24 hour urine for proteinuria and creatininuria. Values of >0.5 g/ day will be considered abnormal. We will measure the time-average proteinuria (TA-P) (52) and the slope of proteinuria (53).

Serum creatinine, proteinuria, age and hypertension have a lower weight when histological lesions are not considered. This finding highlights the importance of the kidney biopsy, not only for diagnosis but for personalized treatment.

-Blood hypertension is defined when values are > 130/80 mmHg or in subjects treated with anti-hypertensive drugs. Treatment will also be conducted in hypertensive patients with blood pressure >130/80 and daily proteinuria more than 0.5 g. Sodium restriction is necessary.

### 6.2 Allocation

After initial eligibility based on screening of the clinical data and kidney biopsy report, each patient will receive an explanation of the trial plan. After the signature of the consensus participation the patient will be enrolled and randomized separately in each trial (allocation 1 to 1 ratio) via a web-based program generating random numbers to ensure allocation concealment. Stratified randomization will be done by age and sex in renal centers. The follow-up study to measure the outcomes will consist of regular visits at the prescribed times to collect clinical and laboratory data and information on drug adherence. Before the trial is complete, each patient will receive a final outpatient visit.

### 6.3 Blinding

Allocation concealment will not be blinded to the group of the assignment.

### 6.4 Statistical Methods

Quantitative variables will be summarized in the presence of normal distribution values as mean+/- SD or median, and interquartile ranges in the presence of non-normally distributed variables. The between-arms comparisons of continuous variables will be performed by independent t-test or Mann-Whitney U test, as appropriate. The between-arms difference of the primary end point (i.e. in the between-arms difference of delta 24h proteinuria from baseline to 6 months in the ACIgAN trial and the between-arms difference of delta 24h proteinuria from baseline to 12 months in the CHRONIgAN trial will be expressed as mean difference and 95% CI. Categorical variables will be presented as absolute and percentage frequency. Proportions will be compared using the Pearson's $X^2$ test or Fisher's test, as appropriate. The time to event analysis will be performed by the Kaplan-Meier curves by comparing the experimental and control group by the log rank test. Multivariate analysis will be based on Cox's regression proportional hazard method and used to assess the relative risk associated with possible baseline prognostic factors such as sex, age, serum creatinine, eGFR, hypertension, histological lesions and therapy.

The primary analysis will be conducted according to the intention-to-treat approach in all randomized patients, irrespective of adherence to the assigned treatment. A per protocol analysis will be also performed. A secondary analysis by linear mixed models (LMM) or generalized estimating equations (GEE) of repeated measurements of eGFR and proteinuria will be also performed. The choice between the two analyses will closely depend on the distribution type of the outcome variables in our study cohorts.

In these models, data will be expressed as regression coefficients, 95% CIs and P value. All analyses will be performed using SPSS for Windows, 17.0 (SPSS Inc., North Sydney, Australia) and STATA/IC 13.1 for Windows (College Station, Texas, US). A *P* value less than 0.05 will be considered statistically significant.

### 6.5 Analysis populations

The primary trial efficacy analysis will be done in the intention-to-treat (ITT) population, including all randomly assigned participants.

A secondary analysis will be performed in the persistent population (PP, i.e. including all patients who persisted on the investigational drugs for at least 4 and 8 months (ACIgAN trial) or 10 months (CHRONIgAN trial).
Patients who need to change their study treatment prior to 1st month (ACIgAN trial) or 4 months (CHRONIgAN trial) will be considered as dropouts.

The safety population will be represented by all patients receiving at least one dose of the investigational drugs.

A recent systematic review (15) evidenced that patients with histological lesion M1 were responsive to corticosteroids. Therefore, we will do a subgroup analysis to evaluate the effect of corticosteroids in active and chronic IgAN patients with lesion M1 versus M0.

## 7. DATA MANAGEMENT

### 7.1 Data collection

Data collection will be done by the participant renal units. To reduce the withdrawn or lost to the follow-up quarterly meetings will be done by webinars.
The designated investigator staff will enter the data required by the protocol into the electronic Case Report Forms (eCRF). Investigator site staff will not be given access to the eCRF until they have been trained.
The Principal Investigator is responsible for assuring that the data entered into eCRF is complete, accurate, and that entry and updates are performed in a timely manner.

### 7.2 Data management

The processes of acquiring and recording data will be done by the eResult Company through the OMNIACARE platform and the ReCaS data center.

The OMNIACARE will be responsible for the acquisition of data from laboratory and measurement devices and will transfer data to ReCaS data center for their processing. The structure is cloud-based and adopts the HAAS model so that it does not depend on a specific cloud service provider and allows an effective scalability of resources. The activity will consist in transporting data from the devices where they are generated to the computing platform. Solutions will be studied and implemented to enable transparent, efficient and reliable transfer of data from their devices at the laboratory participants in the study to the ReCaS data center where the computing platform are located. Remote access to data will be done by means cloud technologies and secure protocols such as WebDAV and

HTTPS. In addition, Dropbox-like personal storage solution will be deployed, in order to allow, store and manage data, sharing them with partners in a simple way.

### 7.3 Data monitoring

An Independent Data Monitoring Committee will carry out interim analyses every 12 months. Data will be presented to the Trial Steering Committee. The interim analysis will be performed on the primary end point when 50% of patients will be randomized and completed 6 months of follow-up. The interim analysis will be done by the Coresearch Agency that is an independent statistician company, blinded for the treatment allocation.

In presence of therapy benefit or futility in the experimental arm, the Independent Data Safety Monitoring Board Committee will report to the Central Ethics Committee the interruption of the study.

### 7.4 Harms

Safety information will be collected for the intention-to-treat population by recording severe adverse events (SAEs). Upon confirmation, the severity of the AEs will be evaluated for the procedures conducted, the outcomes and the relationships to the study drugs. The AEs will be reported to the principal investigator who will confer with an independent monitoring committee whose members will not know the trial-group assignment. The monitoring committee will evaluate the trial safety and, independently, will determine any necessary revisions to the trial design and will validate all decisions for continuing the trial.

### 7.5 Site monitoring and audits

Before study initiation, at a site initiation visit or at an investigator’s meeting, Sponsor personnel (or designated CRO) will review the protocol and the eCRFs with the investigators and their staff. During the study, the field monitor will check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol to Good Clinical Practice, the progress of enrollment, and to ensure that study treatment is being dispensed and managed according to specifications. Key study personnel will be available to assist the field monitor during the visits/webinars. The investigator will maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data and the results of any other tests or assessments. All information recorded on eCRFs will be traceable to source documents in the patient's file. The investigator will also keep the original signed informed consent form (a signed copy will be given to the patient). The investigator will give the monitor access to all relevant source documents to confirm their consistency with the eCRF entries. The Sponsor monitoring standards will require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria and documentation of SAEs.

Source data/documents will be available to inspections by the Sponsor or designee or Health Authorities.

# 8. ETHICS AND DISSEMINATION

## 8.1 Research ethics approval

The protocol is subject to approval by the competent Independent Ethics Committee. It will be carried out according to the Declaration of Helsinki (IV adaption), to the ICH Guideline for Good Clinical Practice and the laws and regulations of the country. During the follow-up every patient will be informed about goals, expected benefits and possible risks, and rights to refuse or to withdraw at any time.
The model of informed consent that will be presented to potential participants has been prepared and approved by the Steering Committee.

## 8.2 Protocol amendments

Substantive amendments with protocol identifiers and dates, transparently described in the trial reports, will be submitted to the ethics committees.

## 8.3 Consent or assent

The written material (information leaflet and consent document) will be presented by the clinical investigators of the renal centers to the participants. After the signature of the consensus participation the patient will be enrolled and randomized separately in each trial (allocation 1 to 1 ratio) via a web-based program generating random numbers.

Additional request of consent will be done for the collection of biological samples (blood, urine and kidney tissue) that will be used for the ancillary study “Cutting-edge bio-molecular analysis for precision therapy in IgAN (BIO-D).

## 8.4 Confidentiality

Every enrolled participant will be identified by a personal code connected to the code of the renal centre. Thus, depersonalized data will circulate for the monitoring of the clinical study. Encrypted digital code will be stored by the renal centers. Information about study subjects will be kept confidential and managed under the applicable laws and regulations.

## 8.5 Insurance

The Sponsor has taken out a liability insurance policy covering this clinical trial. This insurance policy is in accordance with local laws and requirements. The insurance of the Sponsor does not relieve the Investigator and the collaborators from maintaining their own liability insurance policy.

## 8.6 Declaration of interests

Clinical and scientific partners do not have conflict of interest in the publication of data.

## 8.7 Access to data

Prior the main publication only the Steering committee will have access to the overall results. Then, all the investigators will have access to the full dataset after a formal request describing the research.

## 8.8 Ancillary and post-trial care

Ancillary project. BIO-D study

### 8.9 Dissemination policy

The plan to disseminate trial results to key stakeholders will be based on the presentation of data to National and International Congresses and on the submission of papers to international scientific journals. Furthermore, dissemination of data will be done by websites. The plan will be discussed with the Steering Committee.

Sharing of data will be established by the Steering Committee.

## 9. ANCILLARY PROJECT

A cutting edge-molecular study will be conducted in a cohort of 120 enrolled IgAN patients with active or chronic/moderate renal lesions to evaluate the molecular effect of personalized therapy (BIO-D)

### 9.1 Background

Immunoglobulin A nephropathy is characterized by recurrent episodes of macrohematuria in concomitance of upper respiratory tract infections or other mucosal infections, or by permanent microhematuria. The diagnosis is based on the presence of IgA deposits in the mesangium of glomeruli. A "score system" is used for the histological analysis of the kidney biopsy (6-8). Five types of renal lesions are scored: Mesangial hypercellularity (M 0,1), Endocapillary hypercellularity (E 0,1), Segmental glomerular sclerosis (S 0,1), Tubular atrophy/interstitial fibrosis (T 0,1,2), and Extracapillary proliferation, crescents (C 0,1,2). IgAN is characterized also by increased serum levels of galactose-deficient IgA1 (Gd-IgA1) (54).

We have studied the gene expression in leukocytes isolated from the blood samples of IgAN patients and evidenced differentially expressed genes (DEGs). Bioinformatic analysis discriminated 210 DEG1s and functional analysis, operated on the top selected genes, demonstrated that WNT-B-catenin and PI3K/Akt pathways were highly activated in IgAN patients (55). Furthermore, DEGs were also found in monocytes isolated from the blood of IgAN patients and primarily involved genes belonging to the apoptosis signaling, mitochondrial dysfunction and TNFR2/1 were found (56). We also focalized the attention on gross hematuria episode in concomitance to mucosal infections and DEGs were involved in interferon signaling and antigen presentation (57).

Recently, we have evidenced specific DEGs that characterize active renal lesions (E1 and C1,2) compared to chronic renal lesions (T1,2) in kidney biopsy specimens of IgAN patients (58). Bioinformatic analysis has identified transcripts for active (DEFA4, TNFAIP6, FAR2) and chronic (LTB, CXCL6, ITGAX) renal lesions that were validated by RT-PCR and IHC. Finally, two DEGs (TNFAIP6 for acute and CXCL6 for chronic renal lesions) were confirmed in the urine of an independent cohort of IgAN patients compared with non-IgAN patients and controls. In conclusion, we have discovered two urinary biomarkers that may be used for specific clinical decision making during the clinical course of the disease.

Recently, we have obtained preliminary data on gene expression in microvesicles isolated from the urine of patients with IgAN (unpublished data). This means that we have set-up all techniques for the study of transcriptomics in the three compartments of the body (blood, kidney, urine) in IgAN patients with active renal lesions at kidney biopsy and in IgAN patients with chronic renal lesions enrolled in the CLIgAN study.

In conclusion, the CLIgAN study includes a transcriptomic study carried out on the three compartments (blood, kidney and urine) of the IgAN patients at the time of the kidney biopsy and during the follow-up. We will analyze transcriptome changes of peripheral blood mononuclear cells and urinary microvesicles during the clinical course of the disease separately in the two subgroups of patients. This scientific approach will allow a finer molecular stratification of patients and the

identification of specific diagnostic biomarkers that could be used for a precision therapy in IgAN patients.

### 9.2 Aim

Aim of this research project is to integrate transcriptomic results obtained from all three compartments (blood, kidney and urine) in the same patient at the time of the kidney biopsy and then during the follow-up to evaluate the benefit of corticosteroids and the benefit of Dapagliflozin combined with RASBs in patients with active renal lesions and RASBs in patients with chronic or moderate renal lesions. We will analyze transcriptome changes during the clinical course of the disease separately in the two subgroups of IgAN patients. Blood and urine samples will be taken on the same day. This scientific approach will permit a finer molecular stratification of IgAN patients and the identification of specific biomarkers that could be exploited for the identification of novel therapeutic targets and the benefit of personalized therapy.

### 9.3 Objectives

- To obtain a finer molecular stratification of patients with active and moderate/chronic renal lesions.
- To identify specific diagnostic biomarkers that could be used for precision therapy in IgAN patients.

### 9.4 Endpoints

Biomarker analysis on patients' biological samples evaluating:

<u>Blood samples</u>

- the measurement of IgA, Gd-IgA1, Gd-IgA1-specific IgG, Gd-IgA1-specific IgA, Gd-IgA1-IgG immune complexes.
- gene expression pattern in PBMCs from patients with active or chronic/moderate renal lesions

<u>Urine samples</u>

- transcriptomic analysis in urinary cells
- transcriptomic analysis on isolated microvesicles

<u>Tissue sample</u>

- transcriptomic pattern on RNA extracted from kidney tissue in patients with active or chronic/moderate renal lesions

### 9.5 Collection of biological samples

In this project we will collect blood, kidney biopsy and urine samples from IgAN patients with acute renal lesions enrolled in the ACIgAN clinical study and from IgAN patients with chronic and moderate renal lesions enrolled in the CHRONIgAN trial. Blood and urine samples will be collected

in every patient on the same day before kidney biopsy will be performed and then during the clinical course of the disease. IgAN patients will be accurately stratified by the MEST-C classifications.

Blood and urine samples will be collected at different time (T) from patients during the follow-up of the CLIgAN study:

i) patients with active renal lesions: T0,T1,T2,T4,T6, T8,T12,T24, T36 months and
ii) in patients with chronic and moderate renal lesions: T0,T1,T4,T8,T10,T14,T24,T36 months.

Blood samples. 10 ml of blood will be collected in PAXgene tubes for the isolation of peripheral blood leukocytes that will be used for transcriptomic studies.

Serum samples will be collected for the measurement of IgA, Gd-IgA1 and other biomarkers. Some of these biomarkers have been described as predictors of clinical outcome but none has been validated in prospective clinical studies. Therefore, we will measure some current biomarkers involved in the pathogenesis of the disease as Gd-IgA1 during the follow-up.

Urine samples. 30-50 ml of urine of the second morning urination will be collected for the isolation of urinary microvesicles.

Renal biopsy will be used for histological diagnosis and immunofluorescent technique. Then, transcriptomics will be performed in RNA eluted from 5 micron thickness sections for a total of 8 slides.

### 9.6 Methods

All samples will be stored in freezer at -20° or -80°C in the participant renal centers. Every six months, or when possible, the collected biological samples will be shipped to the Schena Foundation for the molecular study. Shipping fees will be supported by the Foundation.

Transcriptome analysis will be done using single cell or nuclear cell RNA-sequencing.

### 9.7 Statistical analysis

Data from transcriptomics will be elaborated by computational analysis and artificial intelligence (artificial neural networks and deep learnings).

Integrative analysis of transcriptomic data will be done combining response to the combined therapy (Corticosteroid and RASBs) in IgAN patients with active renal lesions and to the combined therapy (Dapagliflozin and RASBs) in patients with chronic or moderate renal lesions.

### 9.8 Consent or assent

Additional request of consent will be done for the collection of biological samples (blood, urine and kidney tissue) that will be used for the ancillary study “Cutting-edge bio-molecular analysis for precision therapy in IgAN (BIO-D).

### 9.9 Where the research will be conducted

Biological samples will be collected in the nephrology centers and analyzed at laboratories of Schena Foundation. Every six months the collected biological samples will be shipped to the Schena Foundation for the molecular study. Shipping fees will be supported by the Foundation.

The Schena Foundation is located in the Department of Emergency and Organ Transplantation, Policlinic, University of Bari, which has multiple fully equipped modern molecular biology research laboratories containing analytical and genomic facilities in order to perform all the analysis. Single cell RNA-sequencing (sc RNA-seq) and computational analyses will be done in collaboration with the Department of Molecular Biology of the Bari University.

### 9.10 Dissemination policy

The plan to disseminate trial results to key stakeholders will be based on the presentation of data to National and International Congresses and on the submission of papers to international scientific journals. Furthermore, dissemination of data will be done by websites. The plan will be discussed with the Steering Committee.

Sharing of data will be established by the Steering Committee.

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