Algemene gegevens / General Information

Programma / Programme	:	**COVID-19 Programma**
Subsidieronde / Subsidy round	:	**Bottom-up ronde COVID-19 aandachtsgebied 1**
Projecttitel / Project title	:	**Attenuating the COVID-19-induced organ damage by managing SARS-Cov-2-induced danger signals.**
Projecttaal / Project language	:	**Engels / English**
Geplande startdatum / Planned start date	:	**29-06-2020**
Geplande duur / Planned duration	:	**24 maanden / months**
Datum indienen / Date of application	:	**14-05-2020**
Projecttype / Project type	:	**Toegepast onderzoek**
Vervolg eerder ZonMw-project / Continuation previously funded project ZonMw	:	**Nee / No**

Projectleden / Project members

Prof. dr. P. de Vos (Main applicant)
Functie / Position: Professor | *Opleiding / Education:*
Studierichting / Subject:
T: 06 (10)(2e) | *F:* | *E:* (10)(2e) @umcg.nl

Universitair Medisch Centrum Groningen
Pathologie en Medische Biologie
Sectie Medische Biologie
Postbus 30001
9700 RB GRONINGEN

(10)(2e)
Functie / Position: Postdoctor | *Opleiding / Education:*
Studierichting / Subject:
T: 06 (10)(2e) | *F:* | *E:* (10)(2e) @umcg.nl

Universitair Medisch Centrum Groningen
Pathologie en Medische Biologie
Sectie Medische Biologie
Postbus 30001
9700 RB GRONINGEN

(10)(2e)
Functie / Position: Department administrator | *Opleiding / Education:*
Studierichting / Subject:
T (10)(2e) | *F:* | *E:* (10)(2e) @umcg.nl

Universitair Medisch Centrum Groningen
Pathologie en Medische Biologie
Sectie Medische Biologie
Postbus 30001
9700 RB GRONINGEN

(10)(2e)
Functie / Position: Medical Doctor | *Opleiding / Education:*
Studierichting / Subject:
T: (050) (10)(2e) | *F:* | *E:* (10)(2e) @umcg.nl

Universitair Medisch Centrum Groningen
Intensive Care Volwassenen
Postbus 30.001
9700 RB GRONINGEN

Projectgegevens / Project information

Aandachtsgebieden / Focus

1.1 Thema's aandachtsgebied 1

- Behandeling
- Virus, immuniteit, immuunrespons en pathogenese

Samenvatting / Summary

The goal of this proposal is to test and apply clinically approved drugs (dipyridamole, regadenoson) that attenuate the pro-inflammatory responses against alarm molecules released from damaged cells in mild COVID-19 patients to prevent the progression of the disease. Progressed COVID-19 is associated with a "cytokine storm" induced by the virus and by damage-associated molecular patterns (DAMPs) signals. DAMPs are proinflammatory molecules that can be sensed by toll-like receptors (TLRs). These receptors are differentially expressed in females and males, possibly explaining the higher susceptibility of males for severe COVID-19. DAMPs, cytokines and adipokines are elevated in elderly and in obese individuals, which might explain the higher risk for severe COVID-19. The DAMPs-induced effects can be reduced by adenosine. Adenosine signaling reduces inflammation by inhibition of the NF-??B pathway, an important factor in the production of cytokines. Adenosine signaling enhancers, such as dipyridamole have shown efficacy in COVID-19 patients. Current insight is that adenosine signaling enhancement is a promising, fast and sound approach to reduce DAMPs/TLRs signaling to limit the progression into severe COVID-19 stages. We will study the anti-inflammatory effect of clinically used adenosine enhancers in inflammation induced by SARS-Cov-2 and by different DAMPs in non-infected and infected high-risk groups. Moreover, we will study in a high throughput fashion the levels of DAMPs, adipocytokines and cytokines in >300 plasma samples from different COVID-19 stages collected in The Netherlands. At 6 months from the start of the project, we will perform a randomized human pilot to determine whether the adenosine enhancement with clinically approved drugs prevents the severity of COVID-19. Our approach will contribute to better understanding of the pathophysiology and reduce hospitalization of COVID-19 patients, attenuating the economic and social impact of the pandemic.

Trefwoorden / Keywords

COVID-19, treatment, DAMPs, proinflammation, adenosine signaling, approved drugs

Samenwerking / Collaboration

Samenwerking tussen onderzoek en praktijk / Cooperation between research and practice:

Ja / Yes

Organisaties

Amphia Ziekenhuis Breda
Intensive Care

Universitair Medisch Centrum Groningen
Intensive Care Volwassenen
Postbus 30.001
9700 RB GRONINGEN

Inhoud / Content

Disciplines / Disciplines

- Immunologie, serologie / Immunology, serology

Financiële gegevens / Financial data

ZonMw budget

	Jaar / Year								
Kostenpost	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	**Totaal / Total**
Personeel	(10)(1c)								
Materieel									
Implementatie									
Apparatuur									
Overig									
Totaal / Total									

Co-financiering / Cofinancing

Naam co-financier / Name of cofinancier	Bedrag / Amount	Status

Bijzondere gegevens / Additional information

Vergunningen / Permits

	Verklaring nodig / Statement required?		Status verklaring / Statement status		
	Ja / Yes	Nee / No	Verkregen / Acquired	Aangevraagd / Applied	Nog niet aangevraagd / Not applied yet
METC	X				X
DEC		X			
WBO		X			

Onderschrijvingen / Assents

	Ja / Yes	Nee / No	N.v.t. / N.A.
Code biosecurity / Code Biosecurity			X
Code openheid dierproeven / Code Transparency of Animal Testing			X

Andere vergunningen / Other permits

AANVRAAGFORMULIER PROJECTIDEE – BOTTOM-UP RONDE

COVID 19 programma

Deadline voor indiening: 14 mei 2020 (14:00 u)

LEES ALSTUBLIEFT ALLE INSTRUCTIES IN BIJLAGE "TOELICHTING INDIENING PROJECTIDEE" VAN DE OPROEPTEKST ZORGVULDIG!

Wanneer u het formulier heeft ingevuld:

1. **Zet het formulier om naar een PDF file en controleer de details**
2. **Upload het complete formulier als een bijlage bij uw indiening in Projectnet (Let op: dit zijn twee verschillende links, gebruik maar 1 van de 2!)**

ProjectNet: Aandachtsgebied 1 (voorspellende diagnostiek en behandeling)
ProjectNet: Aandachtsgebied 2 (zorg en preventie)

NAAM VAN DE HOOFDAANVRAGER:

Prof dr Paul de Vos
Co-aanvragers:
(10)(2e)
(10)(2e)
Prof. J Lakey (Department of Surgery, University of California)

ORGANISATIE:

Department of Pathology and Medical Biology, University Medical Center Groningen (UMCG), Groningen, The Netherlands

PROJECTTITEL:

Attenuating the COVID-19-induced organ damage by managing SARS-Cov-2-induced danger signals.

DATASTEWARD:

Wie is de datasteward die de open science en FAIR data planning in uw project ondersteunt? Zie de webinars op de ZonMw website om de datastewards te informeren en ondersteunen.

☐ Ik betrek een datasteward bij mijn project:
Naam: Klik of tik om tekst in te voeren.
Instituut: Klik of tik om tekst in te voeren.
E-mail: Klik of tik om tekst in te voeren.
Was aanwezig bij de webinar: ☐ Ja ☐ Nee

☒ Ik heb nog geen datasteward.

ONDERZOEKSVOORSTEL max 3 pagina's A4 (inclusief literatuurreferenties)	(voorpagina met basisgegevens niet meegerekend - font type Arial 10 pts)

1. PROBLEEMSTELLING EN DOELSTELLING(EN):

The main goal of this proposal is to test and apply clinically approved drugs that attenuate the pro-inflammatory responses against alarm molecules released from damaged cells in mild COVID-19 patients to prevent the progression of the disease. According to the current insight, a local 'cytokine storm' might occur during COVID-19 in organs, such as the lungs, as a consequence of the release of intracellular 'alarm' molecules from cells that are infected and undergo necrosis or necroptosis. These alarm molecules are called danger-associated molecular patterns (DAMPs). This leads, in severe cases, to acute respiratory distress syndrome (ARDS) and multiple organ dysfunction[1]. However, the clinical manifestation of COVID-19 varies from asymptomatic to critical and fatal stages. Just like in other viral diseases, such as in SARS, MERS but also in influenza, males have a higher risk of developing a cytokine storm and severe forms of the disease[1], likely due to a higher expression of Toll-Like Receptor (TLR)4, an important receptor for DAMPs[2]. Obesity and aging are other risk factors, likely due to the imbalance of pro and anti-inflammatory adipokines, such as leptin, visfatin and adiponectin, and higher circulating levels of cytokines such as interleukin-6 (IL-6) (crucial cytokine in COVID-induced cytokine storm), in these individuals.

Searching for **readily available therapies** to prevent the cytokine storm **is urgently needed to reduce mortality and alleviate the current pressures on healthcare and associated socioeconomic consequences.** Characteristics of COVID-19 include increased levels of key proinflammatory cytokines, such as IL-1β, TNFα and IL-6. Production of these cytokines is controlled by pattern recognition receptors (PRRs), such as TLRs. TLRs are activated by DAMPs, triggering NF-κB, an important proinflammatory pathway, inducing the production of cytokines. **Our team has unique knowledge and technologies to study the involvement of these receptors and pharmacological tools to lower the activity of these DAMPs.** SARS-Cov-2 viral RNA likely activates TLR7 and TLR8[3]. TLR-8 induced IL-6 release is inhibited by hydroxychloroquine[4], an emergency use authorized drug for COVID-19, **suggesting a key role of TLRs in the treatment of COVID-19**[5]. **Adenosine** is a nucleoside and an FDA-approved drug, that **strongly inhibits the NF-κB pathway via adenosine receptors activation, reducing cytokine production. Activation of adenosine receptors downregulates TLRs activation, consequently reducing the release of proinflammatory cytokines**, such as IL-6, IL-1β and TNFα[6] in immune cells. Also, in **acute lung injury models, adenosine reduces proinflammation and cytokine release supporting lung integrity**. Our team has unique **knowledge and experience with adenosine as an anti-inflammatory molecule. Regadenoson** (adenosine receptor agonist) **but especially dipyridamole are promising drugs for COVID-19 patients as they both enhance adenosine's anti-inflammatory effects and, additionally, dipyridamole reduces replication of SARS-Cov-2**[7]**. Some efficacy is demonstrated in COVID-19 patients demonstrating the proof of principle of our approach**[7].

Therefore, **adenosine signaling enhancement,** for instance by inhibiting adenosine uptake or using specific adenosine receptors agonists, **is a promising, fast and sound approach to reduce DAMPs/TLRs signaling to limit the progression into severe COVID-19 stages. By applying this, our approach will contribute to reduced hospitalization of COVID-19 patients, reducing the economic and social impact of the pandemic.** Importantly, clinically approved drugs, such as dipyridamole or regadenoson, enhance adenosine signaling and can be rapidly used in COVID-19. These molecules will serve as a benchmark in our studies.

Our project has the following hypotheses:

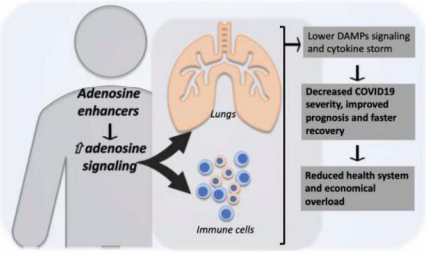
1) DAMPs/TLR activation increases the COVID-19-associated cytokine storm in susceptible individuals (50+ males with overweigh) and 2) adenosine enhancers, such as dipyridamole or regadenoson, reduce the severity of COVID-19-associated cytokine storm improving the clinical outcomes.

We, therefore, aim:

1) To evaluate the anti-inflammatory potential of adenosine signaling enhancement in COVID-19 high-risk populations (50+ males with overweight).

2) To investigate the effects of adenosine signaling enhancement in SARS-Cov-2 and DAMPs-induced inflammation in COVID-19 patients.

3) To prove that pharmacological adenosine signaling enhancement improves COVID-19 clinical outcomes.



2. PLAN VAN AANPAK:

1) *Adenosine enhancers reduce TLR-dependent proinflammatory cytokines in peripheral blood mononuclear cells (PBMCs) dependent on adenosine receptor signaling.*

Here we will answer the question of how different adenosine signaling enhancers can prevent SARS-Cov-2-induced dysregulations in cytokine release by using PBMCs of high-risk populations or patients with severe or mild symptoms. For this, PBMCs from non-infected donors from high- and low-risk populations for severe COVID-19 (i.e. males, obese, 50+ vs. females, normal weight, young) will be stimulated with agonists of different TLRs involved in viral and damage signal responses and with SARS-Cov-2 peptides from our virology department. The different *in vitro* stimulations will be performed in the presence of approved adenosine enhancers, such as (combinations of) dipyridamole or regadenoson, and caffeine (adenosine receptors inhibitor). To induce a cytokine storm, we will use (combinations of) known DAMPs (e.g. mitochondrial (mt)DNA) or molecules known to induce influenza-induced cytokine storm (e.g. TNFα, IL-6, IL-33)[8] and SARS-Cov-2 peptides. After stimulation, we will apply Luminex assays to study crucial cytokines associated with COVID-19 severity[1], such as IFN-γ, IL-2, IL-6, IL-10, IL-1β, TNFα. In a second stage, a similar approach will be applied to PBMCs from COVID-19 patients from our IC (late stage, non-COVID-related IC patients will serve as control) and from UMCG employees that are tested positive (mild symptoms, healthy age-weight-gender individuals will serve as control). Transcriptomics and proteomics approaches will be applied to gain insight into processes and mechanisms behind COVID-induced changes in PBMCs and selected monocyte and T-cell populations in the absence or presence of adenosine (enhancers).

2) DAMPs in plasma/serum of COVID-19 patients

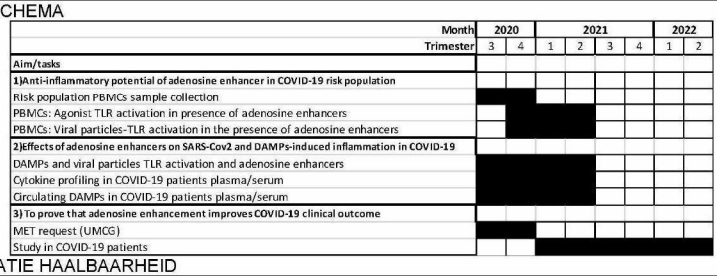
Simultaneously we will study which DAMPs/cytokines/adipokines are released and how they associate with the severity of COVID-19 in different age-weight-gender categories. For this, we will use serial plasma samples (3 per week) from patients admitted directly to the ICU in the UMCG and Amphia (Breda) (>300 samples in biobank) but also from UMCG-employees that are tested routinely and found positive and are being followed up (> 40, 3 times per week sampled). We will quantify the presence of DAMPs, such as HMGB1, ATP, mitochondrial and nuclear DNA, adipokines (e.g. vifastin, adipsin, leptin) and cytokines (IL-6, TNFα) with Luminex. Data such as age, sex, BMI and COVID-19 severity will be registered and stored anonymously.

3) Proof of concept: Randomized controlled pilot study in COVID-19 patients

To prove our hypothesis (start at 6 months from project starting), COVID-19 patients (with mild or non-severe symptoms), that are hospitalized receiving supportive care in the general ward, will be voluntarily enrolled and treated with standard therapy (ST) or ST plus an effective adenosine enhancer (ST+adenosine) for the duration of the disease and will be monitored in the hospital for possible adverse events. Samples from healthy (age-weight-gender matched) will serve as controls. We will initially include 20 patients in each arm (ST or ST+adenosine) until data becomes available to allow a power calculation. Also, if effectiveness is shown, we will include patients from the IC-UMCG under standard IC management (ST-IC) (as control) and ST-IC+adenosine. Blood samples will be taken every three days. Additionally, weekly nasopharyngeal swabs for viral RNA detection will be included. Blood samples will be sent to our clinical laboratory for hemogram, coagulation test, biochemical profile and ultra-sensitive CPR. These tests will give us information about the general health status of the patients. With Luminex, we will evaluate the levels of different cytokines, such as IFN-γ, IL-2, IL-6, IL-10, IL-1β, TNFα, MCP-1, and IL-8 and DAMPS, such as mtDNA or formyl peptides (e.g. Aβ42). Moreover, we will evaluate different leukocytes sub-populations in blood and also, we will include functional parameters, such as neutrophils/NET production in comparison with healthy individuals and mild COVID-19 patients. To assess immunization, we will also measure immunoglobulins (IgG, IgM) against SARS-Cov-2 in the different study groups. Lung CT scans or X-rays will be taken. Clinical progression will be registered and evaluate by our collaborators of the medical staff.

3. HAALBAARHEID VAN HET PROJECT:

TIJDSSCHEMA



MOTIVATIE HAALBAARHEID

We propose the **enhancement of adenosine signaling, with clinically approved and readily applicable drugs, to reduce the DAMPs-induced cytokine storm in COVID-19 to prevent or attenuate severe COVID-19 stages, expediting the recovery phase and reducing health-care cost and hospitalization of vulnerable individuals.** We have extended and unique experience/knowledge on DAMPs signaling and adenosine-induced attenuation of cytokines. Also, we have specific technical facilities to study in a high-throughput fashion the efficacy of molecules on PBMCs and plasma samples. We are collaborating with clinical practitioners within the University Medical Center Groningen (UMCG), Amphia (Breda) and other medical centers from The Netherlands and with an international group of biomedical experts in the field of DAMPs, including University of California Irvine (USA), that also have other patient populations available allowing expansion of our therapies to other countries. Each member of our team will contribute from their expertise, leading to a fast but sound research plan that **will add to the understanding and treatment of COVID-19 reducing the health care and economical effects of the pandemic at a large scale**.

4. RELEVANTIE VOOR DE PRAKTIJK:

Onderbouw de relevantie aan de hand van de in de subsidieoproep benoemde relevantiecriteria

Our proposed idea involves the test and application of available approved adenosine enhancers to be readily applied in COVID-19 with still mild symptoms locally in the Dutch health system (cri. 1) but also, worldwide (cri. 6). We propose a novel idea, supported and built up by our research team's unique knowledge. Therefore, a similar approach elsewhere is not expected (cri. 2). We can generate by that, a unique and worldwide applicable approach, positioning The Netherlands as a pioneer in the COVID-19 treatment (cri. 3). However, we currently do not count on any funding. Therefore, we depend on the financial support of the public sector (cri. 4). Our findings will be published in open access journals. Also, our research will be communicated to the community through local and international press and social media (criteria 5). In our team we included biomedical scientists and clinicians, including clinical care (cri. 8), facilitating translation into clinical practice adding value to our research (cri. 6). This research will also be a proof of principle for future pandemics (cri. 5). We add value to clinically approved drugs that might prevent the severe health consequences of COVID-19 (cri. 8). All this will directly benefit the patients and reduce the economic burden produced by the pandemic. We expect to reduce the severity of COVID-19 leading to a faster recovery of the patients, reducing the long-term consequences that the severe stages that are associated with COVID-19 (cri. 9). The therapy will be sustainable as even if a vaccine is developed, in many susceptible individuals' vaccination will be less efficacious (e.g. elderly) (cri. 5).

5. DEELNAME VAN DE STAKEHOLDER(S) (e.g. patiënten, zorgprofessionals, etc.):

Especially patients with mild COVID-19 symptoms will be the direct beneficiaries of this research. To reach out to professionals and society we will produce a trimestral newsletter and we will organize public open talks to report the progress of the research to the patients/society. The intensive care (IC) staff is also an important stakeholder. The IC staff will participate directly in the collection of blood, establishing the link with biobanks, and in the intervention of COVID-19 patients. We will constantly inform them, through newsletters and seminars, about the progress of our research. We will keep close contact with the Communication Department from our institution (UMCG) and give press releases regularly, this will help us to spread the findings through websites, regional and national media.

6. LITERATUURREFERENTIES (optioneel):

1. Chen, N. *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* **395**, 507–513 (2020).
2. Aomatsu, M. *et al.* Gender difference in tumor necrosis factor-α production in human neutrophils stimulated by lipopolysaccharide and interferon-γ. *Biochem. Biophys. Res. Commun.* **441**, 220–225 (2013).
3. Kawasaki, T. *et al.* Toll-like receptor signaling pathways. *Frontiers in Immunology* vol. 5 461 (2014).
4. Figueroa-Lozano, S. *et al.* Inhibitory Effects of Dietary N-Glycans from Bovine Lactoferrin on Toll-Like Receptor 8; comparing efficacy with chloroquine. *Front. Immunol.* **11**, 790 (2020).
5. Chen, Z. *et al.* Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. *medRxiv* 2020.03.22.20040758 (2020).
6. Pinhal-Enfield, G. *et al.* An angiogenic switch in macrophages involving synergy between toll-like receptors 2, 4, 7, and 9 and adenosine A2A receptors. *Am. J. Pathol.* **163**, 711–721 (2003).
7. Liu, X. *et al.* Potential therapeutic effects of dipyridamole in the severely ill patients with COVID-19. *Acta Pharm. Sin. B* (2020).
8. Liu, Q. *et al.* The cytokine storm of severe influenza and development of immunomodulatory therapy. *Cellular and Molecular Immunology* vol. 13 3–10 (2016).