

Per-operative treatment of post-operative complications (POC)

Technical field

The invention relates to the field of post-operative complications (POC) that are undesirable consequences of surgery. POC forms a major area of concern that adversely affects the quality of surgical care and patient safety. POC range from seemingly minor incidents which resolve without any harm to more serious incidents which require medical treatment, may pose a threat to life, need multiple interventions, increase (third party) hospital and treatment costs, and may at times cause disability or death. In addition to the physical harm caused to patients, surgical complications can lead to psychological stress and worsen the quality of life.

Background

As reviewed by Dencker et al [1], the total volume of major surgeries performed annually worldwide was reported to be 312.9 million in 2012, of which around 40 to 50 million in USA and 20 million in Europe [2]. It is estimated that 1–4% of patients with major surgery will die, up to 15% will have serious post-operative morbidity, and 5–15% will be readmitted within 30 days. An annual global mortality of around 8 million patients places major surgery comparable with the leading causes of death from cardiovascular disease and stroke, cancer, and injury. In a recent study [3], post-operative complications were observed in 31.5% of patients; minor complications (Grade I and II) in 19.75% and major complications (Grade III and IV) in 8.0% of patients. Post-operative mortality (Grade V) was 3.75%. Significant risk factors were the pre-operative presence of comorbidities, higher American Society of Anesthesiologists (ASA) grade, higher BMI, emergency surgery, open surgery, palliative surgery, deeper cavity surgery, on-pump cardiac surgery, higher intra-operative blood loss, prolonged surgical duration, intra-operative complications, and contaminated surgical wounds. Post-operative complications significantly prolonged the hospital stay. A review of literature on post-operative complications in general surgery patients [4] reported an incidence of complications of 5.8-43.5%, with mortality ranging from 0.79% to 5.7%. The review reported an overall complication rate of 37%. In the individual level, the occurrence of POC after the body's stress response to surgical trauma (the surgical stress response [5])

can have a major medical impact on the individual patient, potentially resulting in a decline of both quality of life as well as functional performance.

- The stress response to surgery is a pattern of physiological and pathophysiological changes that occur in response to the stimulus of surgery. The response consists of two broad categories: (i) neuroendocrine–metabolic response, (ii) inflammatory–immune response. The magnitude, invasiveness, and duration of surgery are central in determining the degree of the body's integrated stress response. Major open vascular and abdominal surgery, joint replacement surgery, and cardiac surgery using on-pump cardiopulmonary bypass (CPB) elicit the greatest surgical stress response [5].
- 10 Payers and regulators are also interested in patient outcomes and quality improvement. From a societal standpoint, POCs account for a large financial burden in the form of additional health care expenses, thereby increasing US healthcare costs in an estimated order of magnitude at around \$42.000,- (at price levels 2016; at around \$55.000,- when adjusted for US inflation at price levels 2024) in additional healthcare expenses per patient
- 15 [6]. Many health organizations and the Centers for Medicare and Medicaid Services (CMS) use pay for performance and are now starting to withhold payment for complications deemed preventable to improve outcomes [7]. Khuri et al [8] already show that events in the post-operative period are more important than pre-operative patient risk factors in determining survival after major surgery. Specifically, the occurrence of a complication
- 20 within the first 30 days post-operatively, independent of the patient's pre-operative risk, reduced median patient survival by 69% in the total patient study group.
- Increased costs of surgical complications have been borne mostly by third-party payers. However, numerous policy changes aimed at incentivizing high-quality care shift more of this burden to hospitals. The potential effect of these policies on hospitals and payers is poorly
- 25 understood. Healy et al [6] evaluated costs associated with surgical quality and the relative financial burden on hospitals and payers and conclude that hospitals and third-party payers experience increased costs with surgical complications, with hospitals experiencing a reduction in profit margin. Both hospitals and payers appear to currently have financial incentives to promote surgical quality improvement. Roach et al [9] divides variable costs
- 30 (defined as expenses that vary with the level of patient care, such as nursing labor and drug costs), and fixed costs. From a breakdown of median costs between hospital stays with and

without POC it is concluded in Roach et al that the ratio of variable costs : direct costs ~ 2:1. Studies from Australia [10] as well as from Europe [11] show that major and treatment-dependent POC (Clavien-Dindo scales II/IV) weigh most heavily on increasing costs with a ~250% rise over costs associated with patients without POC.

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In conclusion, both hospitals and third-party payers experience increased costs with surgical complications, with hospitals experiencing a reduction in profit margin. Both hospitals and payers appear to currently have medical as well as financial incentives to promote surgical quality improvement.

- 10 The main arena of the surgical stress response is the microvascular bed. The vascular system of microcirculation represents the largest vascular surface area of the body and is vital for the effective delivery of oxygen and nutrients to cells and removal of waste products from tissue beds. Following major surgery, patients show a surgical stress response generating microvascular complications with increased capillary leakage, hemodynamic instability, and
- 15 compromised tissue perfusion as clinically relevant consequences. The surgical stress response manifests itself by capillary leakage within the microvascular bed with a heterogeneous ischemia and subsequent re-perfusion of diverse parts of the tissue bed in various organs. This malperfusion elicits distinct DAMP/PAMP signaling towards vascular cells and surrounding tissues. Through this DAMP/PAMP signaling, ischemia-reperfusion
- 20 injury (IRI) may result in a wide array of post-operative clinical complications (POC). Throughout surgery, perfusion parameters of patients are routinely monitored to determine the need for fluid and vasopressor therapy in maintaining proper heart function and resulting microcirculation. This Standard-of-Care (SoC) in managing the surgical stress response may however aggravate the post-operative condition of the patient, in particular
- 25 with fluid overload.

- As reviewed before [12], surgical trauma stimulates a two-pronged response that involve both the nociceptors of the somatosensory nervous system and cytokine-receptors of immune cells, eliciting stress responses that include activation of the hypothalamic–pituitary–adrenal axis, sympathetic nervous system, and immune responses, with main
- 30 effects on the vascular system, directing vascular cells and immune cells to maintain

physiological homeostasis peri-operatively as optimal as possible. Higher invasiveness of surgery with (poly)trauma elicits a higher degree of surgical stress responses that include not only two-pronged nociceptor-mediated neuroendocrine-metabolic and cytokine-mediated inflammatory-immune responses, but also responses generated by damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). The pathological importance of DAMPs/PAMPs signaling is starkly evident in the posttraumatic or post-surgical stress response, wherein vascular complications compromise both resident vascular- as well as immune-cells. DAMP/PAMPs have been identified to trigger specific responses which are associated with the occurrence of major post-operative complications related to the vascular system, decreased tissue perfusion and disseminated-intravascular-coagulation (DIC) effects that further obliterate the vascular system, leading to tissue-ischemia and necrosis throughout the patient.

Prolonged operative time is found [13] associated with an increase in the risk of such vascular complications and tissue damage. For instance, a positive association between the duration of surgical procedures and post-operative adverse events leading to complications such as surgical site infection (SSI), venous thromboembolism (VTE), bleeding, hematoma formation, and necrosis has been reported in prospective and retrospective studies across various surgical procedures. Given the adverse consequences of vascular complications, decreased operative times are thought to be a universal goal for surgeons, hospitals, and policy-makers. However, decreasing operative time may not always warrant maintaining high-levels of surgical care.

Length-of -stay (LOS) in the intensive care unit (ICU) or the hospital is an important outcome as a marker of resource consumption. Determining which factors increase LOS may provide information on reducing costs and improving the delivery of care. Prolonged LOS was found [14] associated with pre-operative, intra-operative, and post-operative factors. Although pre-operative factors were independently associated with a prolonged LOS, the factors found generating pronounced significant ($p < 0.01$) risks for a prolonged LOS were the intra-operative process of care and the occurrence of post-operative adverse events (AE). To reduce costs, efforts should be made to shorten the intra-operative process and to minimize the number of post-operative complications.

The on-pump Coronary Artery Bypass Grafting (CABG) procedure is a major surgical procedure typically performed with the patient attached to a cardiopulmonary bypass machine providing extracorporeal circulation (hence on-pump) and compromising hemodynamic stability of the patient. Early on [15], in cardiac surgery significant differences between patients with and without adverse events were already found. For example, coronary artery bypass graft (CABG) patients with adverse events had prolonged postsurgical LOS (18.5 days +/- 13.2 vs. 13.2 +/- 6.2, $p < 0.001$) and higher mortality rates (15.2% vs. 2.6%, $p < 0.001$). Currently, with improved surgical techniques, with CABG mortality figures have declined, but morbidity and treatment costs from POC remains high and effects patients during their stay in the intensive care unit as well as throughout the full length of stay in the hospital. Overall, the occurrence of AEs after major surgery was also found to lead to substantial excess length of stay in the ICU and in the hospital and increased hospital stay and treatment costs. According to a study from 2002 ([16]) from the US involving 253 geographically distinct US hospitals, daily costs were greatest on intensive care unit day 1 (mechanical ventilation, 10,794 dollars; no mechanical ventilation, 6,667 dollars), decreased on day 2 (mechanical ventilation: 4,796 dollars; no mechanical ventilation, 3,496 dollars), and became stable after day 3 (mechanical ventilation, 3,968 dollars; no mechanical ventilation, 3,184 dollars). As studied in 2021 (Kaiser Health Facts, Hospital adjusted expenses per Inpatient Day by Ownership, 2022) costs per day stay in the hospital in the USA were found for Nonprofit hospitals to be : \$3,013, for For-profit hospitals: \$2,296 and for State/local government hospitals: \$2,742. In The Netherlands in 2015, patients experiencing an AE stayed 5.11 (95 % CI 3.91–6.30) more days in hospital and cost €2600 (95 % CI €1968–€3232) more compared to those without an AE [17]. Cardiac surgery AEs are found among the most frequent and significant contributors to the morbidity, mortality and cost associated with hospitalization [18].

Various peri-operative methods, ranging from carbohydrate loading, probiotics, and early post-operative feeding to post-operative pain-control by opioids or analgesia have been employed to reduce incidence of post-operative complications. Some have shown benefits independently or if incorporated into a multimodal approach, while others have proved counterproductive and hence their use is now discouraged. Parenteral nutritional treatment with L-Alanyl-L-Glutamine (in one-letter-code AQ; sold as Dipeptiven; (ref: Dipeptiven

datasheet) at 1g/kg/day (0.00460 mol/kg/day) before and after surgery (6 ± 2 and 5 ± 1 days pre-, and post-operatively, respectively, and in combination with enteral nutrition with L-Alanyl-L-Glutamine was found to decrease post-operative rates of wound infection, intra-abdominal abscess formation and wound dehiscence in patients operated for colorectal cancer [19]. A systematic review of glutamine dipeptide nutritional supplementation on primary outcomes for elective major surgery [20], showed parenteral nutritional treatment by glutamine supplementation (preferably with L-Alanyl-L-Glutamine or Glycyl-L-Glutamine (in one-letter-code GQ)) given to patients undergoing major elective abdominal surgery to not significantly affect primary clinical endpoints such as mortality, overall morbidity, and the occurrence of infectious complications. Similarly, in clinical trial NCT04560309 ([21]), use of Dipeptiven at 0.50 g/kg/day (0.00230 mol/kg/day) did not reduce ventilator time, intensive care time, nor postoperative use of vasoactive and inotropic nor vasoactive inotropic score. Heyland *et al.*, in the Reducing Deaths due to Oxidative Stress (REDOX) study [22], observed significantly increased in-hospital and six-month mortality rates with said parenteral and enteral use of L-Alanyl-L-Glutamine, without reducing the nosocomial infection rate in ICU patients provided with 0.50 g of the dipeptide AQ per kilogram per day given intravenously and 42.5 g of AQ and GQ dipeptides, which provide 30 g of glutamine, per day given enterally. The dipeptide N(2)-L-alanyl-L-glutamine is endogenously split into the amino acids glutamine and alanine hereby typically supplying glutamine with infusion solutions for parenteral nutritional treatment. The released amino acids flow as nutrients into their respective body pools and are metabolized according to the needs of the organism. Many disease conditions, in which parenteral nutrition is indicated, are accompanied by glutamine depletion, which glutamine containing nutritional infusion regimens may counteract. Pharmacokinetic studies indicate that N(2)-L-alanyl-L-glutamine is rapidly split into alanine and glutamine after infusion. In man, half-lives of between 2.4 and 3.8 (in terminal renal insufficiency 4.2 min) and a plasma clearance of between 1.6 and 2.7 L/min were determined. The disappearance of the dipeptide was accompanied by an equimolar increase of the corresponding free amino acids. Hydrolysis is considered to take place exclusively in extracellular space.

The above analysis [20] questioned a protective effect of such nutritional glutamine supplementation on length of hospital stay in surgical patients, as this observation seems

difficult to interpret for several reasons. Firstly, a shorter LOS may be considered a reliable parameter of recovery and outcome only when consistent with improved morbidity.

Secondly, LOS is trustworthy only when a priori definition of the discharge criteria are clearly stated, and none of the trials examined had this feature. Otherwise, this parameter may be highly dependent on subjective assessment and influenced by non-clinical parameters, such as social and economic conditions, department organization, practice style, and type of primary care provider [23]. Established enhanced-recovery-after-surgery (ERAS) measures apply a mix of patient education, short-acting anaesthetics, pain management, fluid therapy, oral nutrition and early mobilization to improve post-operative recovery [24].

- 10 As hemodynamic instability results in inadequate tissue perfusion and oxygenation, with an inadequate blood supply to an organ or tissue leading to conditions with ischemia followed by reperfusion damage, [25-27], a well-established mainstay of peri-operative care is the use of fluids and vascular-active (vasopressor/inotropic) agents during and after surgery to increase cardiac preload, increase cardiac output and increase vascular tone.
- 15 These agents are typically intravenously applied to the vascular system. Indeed, to ameliorate peri-operative hemodynamic instability, guidelines for standard of care (SoC) in cardiac surgery recommend intravenous fluid therapy and vasopressor/inotropic therapy to improve cardiac preload and tissue perfusion (Wahba et al. 2019; Engelman et al. 2019). Such SoC regimens are established on a per-patient basis governed by the patient's actual
- 20 needs determined through routine monitoring of the patient already during surgery. However, these per-operative SoC-regimens may paradoxically also add to the causes of POCs by affecting hemodynamic stability in multiple organ systems. The volume of fluid administered during the peri-operative period, as well as the amount of vasopressors or inotropic agents provided (inotropic score) may enhance the risk of post-operative organ
- 25 dysfunction. While these treatments may improve the acute consequences of hemodynamic instability, both fluid overload as well as an increased need for hemodynamic support using inotropics and/or vasopressors (reflected in a higher inotropic score) are associated with increased incidence of POCs and increased LoS in the ICU and hospital, and are important predictors of both short-term and long-term mortality [28-31]. Thus, factors deemed
- 30 essential in peri-operative SoC, such as the volume of fluid administered, or the total

amount of vasopressors or inotropic agents provided (inotropic score) may enhance the risk of post-operative organ dysfunction due to increased POC.

Peri-operative organ injury is deemed to be caused primarily by impaired vascular integrity with insufficient tissue perfusion followed by a detrimental ischemic-reperfusion cascade of events or a dysregulated inflammatory response, and represents a major risk factor for post-operative morbidity and mortality in surgical patients [32]. Also in literature, both fluid overload as well as insufficient tissue perfusion were found to associate with increased incidence of POCs and increased LoS in the hospital and are considered important predictors of late mortality [28-33]. In particular, no specific pharmacological therapies have as yet been proven effective in the prevention or reversal of post-operative vascular complications leading to organ injury [32].

Compound EA-230 (tetrapeptide Alanyl-Glutamyl-Glycyl-Valine (AQGV)) is a first in class of a distinct and new class of drugs: autophagy-inhibiting compounds that comprise peptides and/or amino acids that are for at least 50% selected from the group of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R) that target the nutrient sensing system of the mechanistic-target-of-rapamycin (mTOR) and inhibit autophagy, therewith addressing issues of vascular permeability, tissue repair and inflammation [34-36]. Some of which autophagy-inhibiting-peptides (AIP) have also been provided with a specific receptor target motif that allows to target said autophagy-inhibiting compound to specific cells such as resident-vascular cells and/or immune cells. Several of EA-230's therapeutic properties were reflected in a Phase 2b (EASI)-study which demonstrated a significant reduction of Net Fluid Balance (NFB) and LoS in ICU and hospital when EA-230 was given at 90mg/kg/hr (mol/kg/day; kg calculated per individual weight of the subject to be treated) intravenously during the on-pump phase of CABG, from the start of the first surgical incision until the termination of the cardiopulmonary bypass [37-39], while SoC was provided. EA-230 was earlier found to down-regulate PAMP/DAMP signaling [40].

As to earlier, albeit partially, reported EASI-study results shown in WO/2021/206547 and WO/2021/066649 (herein also ref [39]); these two applications do not carry any results or indications of results relating to the incidence or duration of post-operative complications

(POCs) per patient treated. From this lack of understanding on further therapeutic potential of AIP, the applicant of the current application proceeded with a double-blind post-hoc analysis of the existing Adverse Event safety data (measured as adverse events (AE) that, as is standard practice in clinical trials, had not been statistically analyzed yet.

5 Summary of Invention

To further evaluate, the existing Adverse Event (AE) safety dataset from the EASI-study was analyzed in a post-hoc double-blind fashion to assess the possible differences in the manifestation of POC between AIP (EA-230)- and placebo-treated patients. The European Perioperative Clinical Outcome standards for definitions and use of single-organ-outcome-measures (SOOMS) for assessing clinical effectiveness in perioperative medicine [EPCO, 87] were used as clinical outcome measure to assess from the AE-data set the clinical effect of per-operative treatment with AIP on POC after cardiac surgery.

AE data were reduced to bona fide POC data only when matching one of 22 different SOOMS as identified by EPCO. In this way 500 individual adverse events were adjudicated to 175 individual SOOMS (see also table 1). These SOOM data were then graded according to severity of complication into “Mild”, “Moderate” or “Severe”, again following the EPCO standards that allow differential severity grading according to SOOMS and therewith are capable of identifying and measuring incidence and duration of distinct POC per patient as well as determining cumulative incidence of cumulative duration of total POC per patient.

Found AE – and (then unblinded) POC-data were compared and the differences between the two treatment arms were statistically analyzed. A total of 500 AEs were reported, 217 AEs in the EA 230 group (n =90) and 283 AEs in the placebo group (n=89). Said AE-data set was found to be not normally distributed. No significant differences were found between the number of AEs in the placebo group compared to the EA-230 group when analyzed in the Mann-Whitney test.

The double-blinded adjudication led to a first analyses population of 110 patients with 175 SOOMS: 53 patients with 81 SOOMS in the EA-230 arm, and 57 patients with 94 SOOMS in the placebo arm. There is a wide distribution of SOOMS observed which justifies that all SOOMS as defined by EPCO (except SOOM 1.13 that relates to non-cardiac surgery only)

may be relevant in the target population. The number of SOOMs in the EA-230 arm versus the placebo arm was not significantly different.

From the group of 110 patients with a SOOM, the SOOM duration (calculated from start and end date and time) could be established from the AE-data set for a second analysis
5 population of 71 patients, with 98 SOOMS: 32 patients with 42 SOOMs in the EA-230 arm and 39 patients with 56 SOOMs in the placebo arm. Again, the number of SOOMs in the EA-230 arm versus the placebo arm was not significantly different.

The third analysis population was determined on the bases of severity grading and considered only those SOOMs of defined duration with grading “Moderate” or “Severe”
10 according to EPCO definitions or with any of Grade II to V according to Clavien-Dindo definitions. Moderate and severe SOOMs are considered clinical- and patient-relevant, as these reflect the requirement for specific clinical treatment whereas SOOMs classified as mild do “not usually require specific clinical treatment”.

This third analysis comprises 60 patients with 81 moderate and severe SOOMs with defined
15 duration; 30 patients with 37 SOOMs in the EA-230 arm and 30 patients with 44 SOOMs in the placebo arm. Again, the number of moderate/severe SOOMs in the EA-230 arm versus placebo was not significantly different.

Surprisingly, however, in the second and third analyses population with patients having SOOMS with defined duration, SOOM durations as well as cumulated SOOM durations,
20 (herein also identified as cumulated POC duration), per patient with POC, were significantly and strongly reduced.

The median total POC-duration was significantly reduced from 42.9 hrs in the placebo arm to 24.1 hrs in the EA-230 arm ($p=0.0040$, Table -middle column) in the population with a SOOM of defined duration. Moreover, in the third analyses population, the required
25 treatment duration, identified as duration of moderate and severe SOOMS that required medical treatment of a SOOM, as well as cumulated POC duration of required medical treatment of a POC, per patient with POC, were significantly and strongly reduced. For the population with moderate or severe SOOMs of defined duration, the median total POC-duration and duration of medical treatment was significantly reduced from 44.3 hrs in the

placebo group to 17.1 hrs in EA-230 ($p=0.0026$, Table -righthand column). Thus, surprisingly, managing post-operative ischemia-reperfusion injury with pre-operative AIP does not seem to significantly alter the incidence of POC but instead has major significant and beneficial effects on the (cumulative) duration of SOOMS and POC of AIP-treated patients as well as
5 on the duration required for medical treatment of said SOOMS and POC that are considered serious enough to require medical treatment.

The present invention provides a source of autophagy-inhibiting amino acids for parenteral application, preferably intravenous application, preferably intravenous application by infusion with a solution for intravenous administration comprising said source of amino
10 acids, said source selected (preferably at least partly independently selected) from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R), as a medicament for use in at least a per-operative treatment of the vascular system (at least during surgery) to reduce duration, preferably cumulative duration, of a post-operative complication (POC), preferably cumulative post-
15 operative complications in a subject, preferably a human subject, considered or thought considered in need thereof. Preferably, said POC or POCs are definable and/or gradable according to the Clavien-Dindo or EPCO grading systems as discussed herein. Also, the invention provides use of a source of autophagy-inhibiting amino acids for parenteral application wherein each of said amino acids is selected (preferably at least partly
20 independently selected) from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R), characterized in that it is useful for preparing as a medicament suitable for use at least per-operatively for the treatment of the vascular system of a human subject considered in need to reduce duration of possible future post-operative complications (POCs). In a preferred
25 embodiment, said source is an autophagy-inhibiting peptide (AIP). Said AIP is preferably from 2 to 30 amino acids long, more preferably from 2 to 25 amino acids, more preferably from 2 to 20, more preferably from 2 to 18 amino acids, more preferably from 2 to 16, more preferably from 2 to 14 amino acids, more preferably from 2 to 12. In a preferred
30 embodiment, said AIP is preferably from 3 to 30 amino acids long, more preferably from 3 to 25 amino acids, more preferably from 3 to 20, more preferably from 3 to 18 amino acids, more preferably from 3 to 16, more preferably from 3 to 14 amino acids, more preferably

from 3 to 12. In a more preferred embodiment, said AIP is preferably from 4 to 30 amino acids long, more preferably from 4 to 25 amino acids, more preferably from 4 to 20, more preferably from 4 to 18 amino acids, more preferably from 4 to 16, more preferably from 4 to 14 amino acids, more preferably from 4 to 12 amino acids long. In another embodiment, and from the perspective of avoiding (preferably avoiding eliciting in long-time use) B-cell or T-cell immunity, length of such an AIP is not necessarily longer than 9 amino acids, preferably no longer than 8, more preferably no longer than 7 amino acids (not counting possible added targeting motifs as discussed below). In a most preferred embodiment, said AIP comprises 4-6 amino acids.

10 In a further embodiment, a tetrapeptide AIP for parenteral application is preferably administered intravenously via infusion during surgery with a suitable solution applying an administration rate of at least 0.08 mmol/kg/hr, preferably at least 0.12 mmol/kg/hr, more preferably at least 0.16 mmol/kg/hr, more preferably at least 0.24 mmol/kg/hr.

In yet a further embodiment, a pentapeptide AIP for parenteral application is preferably administered intravenously via infusion during surgery with a suitable solution applying an administration rate of at least 0.067 mmol/kg/hr, preferably at least 0.1 mmol/kg/hr, more preferably at least 0.133 mmol/kg/hr, more preferably at least 0.2 mmol/kg/hr,

In yet a further embodiment, a hexapeptide AIP for parenteral application is preferably administered intravenously via infusion during surgery with a suitable solution applying an administration rate of at least 0.054 mmol/kg/hr, preferably at least 0.081 mmol/kg/hr, more preferably at least 0.11 mmol/kg/hr, more preferably at least 0.16 mmol/kg/hr.

For treatment of acute conditions, it is preferred that an AIP according to the invention, in particular when long-term storage is foreseen, is not provided with an N-terminal glutamine while this amino acid at this position may turn into a pyroglutamine, rendering the peptide less well degradable, a condition also known from distinct glutinins. In treatment of chronic conditions, increased stability may be considered an advantage, thus N-terminal glutamine may not need to be avoided.

The invention also provides a method for reducing healthcare costs of post-operative complications (POC) in a subject, said method comprising providing said subject at least during surgery with a source of autophagy-inhibiting amino acids, wherein each of said

amino acids is selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R).

Under 2024 USA-pricing, the study results herewith show that treatment with such a source of autophagy-inhibiting amino acids may provide savings of > \$11.140,- on hospital stay per patient (said savings not including additional savings on medical treatment of POCs). In a preferred embodiment, said source is provided as an autophagy-inhibiting-peptide (AIP). As dipeptides have a very short half-life, it is preferred that said peptide comprises at least 3, preferably at least 4 amino acids. Preferably at least 4 amino acids long peptides are preferred when it is desired to limit peptide uptake via di/tri-peptide PepT1/PepT2 transporters to limit targeted delivery to cells beyond the vascular system. In a preferred embodiment, such a source as provided herein is provided for use to reduce duration of moderate or severe post-operative complications (POCs) in a subject. It is preferred that said POC are identifiable through a grading system according to EPCO or Clavien-Dindo. In a further preferred embodiment, it is preferred that said POC comprise infection. Individual POC are herein also called SOOM (single organ outcome measure). It is especially preferred that said per-operative treatment lasts at least 30 minutes, more preferably at least 60 minutes, more preferably at least 90 minutes, more preferably at least 120 minutes, more preferably at least 150 minutes, more preferably at least 180 minutes, more preferably at least 210 minutes, more preferably at least 240 minutes, more preferably at least 270 minutes, more preferably at least 300 minutes, as for example is shown in table 4 relating to POC of a mild, moderate or severe nature. In another embodiment, it is also preferred that said per-operative treatment lasts at least 95 minutes, preferably at least 156 minutes. As a rule of thumb, a treatment of around 240 minutes often provides sufficient benefit and correlates well with hospital procedures, see for example figure 5. In individual cases longer treatment may be decided. We compared the average gain (= reduction of POC duration in hours) in the set of patients with POC of defined duration. Groups were defined as "short" or "long" according to minutes of treatment with placebo or EA-230 shorter or longer than median operation time which was 154 minutes over the whole population. "Short" treatment showed a gain for active of 4.8 hours (95 minutes to 152 minutes treatment length resulted in POC duration in Placebo of 6.0 hrs versus 1.2 hrs in EA-230) . "Long" treatment showed a gain for active of 7.0 hours (156 minutes to 249 minutes treatment

length resulted in POC duration in Placebo of 8.6 hrs versus in EA-230 of 1.6 hrs) These results indicate distinct benefits of prolonged treatment with EA-230 where the gain translates in a strongly reduced need to provide said patients with a specific medical treatment aimed at treating the specific SOOM in question, and thus of cumulative duration of POCs as a whole.

As also discussed below, in general, there is no distinct advantage to starting the infusion before a surgical stress response is expected, except when a patient is considered to suffer from pre-operative sepsis or from pre-operative injuries, be it from the battlefield or civilian accidents. Septic patients come to the operating room regularly and they need resuscitation and source control. The invention discloses herein that in general, there is no distinct advantage to starting the infusion before a surgical stress response is expected, except when a patient is considered to suffer from pre-operative sepsis or from pre-operative injuries, be it from the battlefield or civilian accidents. Septic patients come to the operating room regularly and they need resuscitation and source control. The present invention further provides the source for use according to the invention wherein said per-operative treatment is followed by a post-operative parenteral treatment (after surgery) with said source, typically wherein said source of autophagy-inhibiting amino acids is not only provided per-operatively but also provided to said subject after surgery, preferably wherein said subject is deemed suffering from a systemic inflammatory response after surgery. It is for example preferred to extend or prolong the intravenous treatment procedure with the autophagy-inhibiting amino acid or peptide solution until after the patient is expected to experience the inflammatory-immune response that often mounts or follows in a second phase of the surgical stress response after the per-operative neuroendocrine-metabolic response so that the patient may also benefit from the beneficial anti-inflammatory effects of said amino acid or peptide solution as well. In certain conditions of surgery, such as in CABG procedures such mounting of an inflammatory-immune response is relatively delayed to a later phase. Then, the anti-inflammatory effects of a solution comprising a source of the present invention may remedy local as well as systemic inflammatory surgical stress responses occurring in that later phase. Preferably such intravenous pre-operative treatment may be extended post-operatively, for example to at least 30, preferably to at least 90 minutes after terminating surgery, preferably at least until said patient has been transferred away from the operating

room and brought to the ICU, and treatment with said solution may be resumed later as well. It is preferred that the source for use according to the invention comprises autophagy-inhibiting amino acids wherein said autophagy-inhibiting amino acids are selected from the group consisting of A, Q, G, V, L and P. In another embodiment, said autophagy-inhibiting amino acids are preferably selected from the group consisting of A, Q, L and P. It is preferred that the source for use according to the invention for use in reducing cumulative duration of post-operative complications (POC), preferably multiple organ POC, in a, preferably human, subject considered or thought considered in need of medical treatment of POC, comprises at least a dipeptide selected from the group AQ, QQ, LQ, GQ, PQ, VQ, AL, LL, QL, GL, PL, PA, VL, AL, PQ, QA, QL, QG, QP, QV, LA, LG, LP, and LV. In another preferred embodiment, it is preferred that the source for use according to the invention for use in reducing cumulative duration of post-operative complications (POC), preferably multiple organ POC, in a, preferably human, subject considered or thought considered in need of medical treatment of POC, comprises at least a tripeptide selected from the group AQG, QQG, LQG, GQG, PQG, VQG, ALG, LLG, QLG, GLG, PLG, VLG, VLA, VLP, PAL, ALP, QAG, QLG, QGG, QPG, QVG, LAG, LGG, LPG, and LVG. In another preferred embodiment, is preferred that the source for use according to the invention for use in reducing cumulative duration of post-operative complications (POC), preferably multiple organ POC, in a, preferably human, subject considered or thought considered in need of medical treatment of POC, comprises at least a tetrapeptide selected from the group AQGV, QQGV, LQGV, GQGV, PQGV, VQGV, ALGV, LLGV, QLGV, GLGV, PLGV, VLG, V, QAGV, QLG, QGGV, QPGV, QVG, LAGV, LGGV, LPGV, VLPA, LPAL, PALP and LVGV. The invention also provides a solution for intravenous administration comprising a source of amino acids for parenteral application selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R), as a medicament for use in at least a per-operative treatment of the vascular system (at least during surgery) to reduce duration, preferably cumulative duration, of post-operative complications (POCs) in a subject.

The invention provides a method of medical treatment (therewith improving on nutritional treatment) for reducing cumulative duration of post-operative complications (POC), preferably multiple organ POC, in a, preferably human, subject considered or thought considered in need of medical treatment of POC, said treatment comprising providing the

vascular system of said subject at least per-operatively (during surgery) with a source of autophagy-inhibiting amino acids selected for at least 50%, preferably for at least 75%, more preferably for at least 90% most preferably for 100% from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R). In a preferred embodiment, the invention avoids the pitfalls of nutritionally overdosing glutamine pre- and post-operatively. It is preferred that POC are identifiable through a grading system according to EPCO or Clavien-Dindo. In a preferred embodiment, the invention provides a method of medical treatment for reducing cumulative duration of moderate or severe post-operative complications (POC) in a subject (POC for example identifiable through a grading system as discussed herein), said treatment comprising providing the vascular system through intravenous infusion of said, preferably human, subject considered or thought considered in need of medical treatment of POC, at least per-operatively, preferably with an infusion fluid (typically a watery (aqueous) solution is used) comprising a source of autophagy-inhibiting amino acids selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R). A preferred source for infusion is a so-called autophagy-inhibiting peptide (AIP) comprising at least 2, preferably at least 3, more preferably at least 4 or 5 autophagy-inhibiting amino acids selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R) that are bound by a common peptide-bond. AIP's effect on capillary leakage with resulting ailments in whole body tissue perfusion and cumulative duration of POC is preferably assessed in multiple organs because capillary leakage occurs systemically and ailments in whole body tissue perfusion affect all organs. The European Peri-operative Clinical Outcome (EPCO) definitions for organ dysfunction (Jammer et al. 2015) may be chosen as a reliable clinical measure. Double-blinded re-analysis of the (Serious) Adverse Events ((S)AEs) in the Phase 2b (EASI) study as provided herein according to the EPCO definitions confirmed the positive effect of AIP on duration and need for medical treatment of POC.

Whereas AIP dipeptides or tripeptides are commonly degraded via extracellular hydrolysis of the peptides to amino acids followed by amino acid influx through amino acid channels into cells, longer AIP peptides, such as tetra – or pentapeptides are at least partly taken up by

cells via common mechanisms of endocytosis. It is herein for example disclosed that per-operative use of AIP AQGV in reducing duration of multiple organ POC is advantageous compared to per-operative use of AIP AQ for that purpose. Where for AQ fast hydrolysis within the vascular system is reported to take place exclusively in the extracellular space, slightly longer autophagy-inhibiting peptides, such as AQG, LQGV, AQGV, VLPLAP or AQGVLP last longer in the vascular environment and are more easily taken up and processed in cells relevant for its autophagy-inhibiting therapeutic effect. Also, in lethal animal models of sepsis [41-43], an-order-of-ten-higher dose level of source of amino acids, when comparing the AIP dipeptide versus the AIP tetra- or longer peptide, respectively, were required for a beneficial effect. The shorter the length of the AIP carrying that source into the vascular system, the more AIP in mg/kg is needed. For a dipeptide, intravenous dosing levels in human subjects are at around 500-1000mg/kg/hr, tripeptides dose at 250-750mg/kg/hr and tetrapeptides at 50-100mg/kg/hr. Preferably, length of such an AIP as provided herein is at least 3 amino acids, more preferably at least 4 amino acids. In a preferred embodiment, said at least 4 amino acids at least comprise A, Q, G and V. In another preferred embodiment, said at least 4 amino acids at least comprise L, Q, G and V. In another embodiment, length of such an AIP is not necessarily longer than 9 amino acids, preferably no longer than 8, more preferably no longer than 7 amino acids (not counting possible added target motifs as discussed below). Suitable examples of somewhat longer AIPs for use in a method according to the invention are LQGVLPALP, AQGVLPALP, LQGVLPAL, AQGVLPAL, LQGVLPA, AQGVLPA, GVLPALP, GVLPAL and VLPALP, and more can be prepared using combinations of dipeptide sequences selected from the AIP group AQ, QQ, LQ, GQ, PQ, VQ, AL, LL, QL, GL, PL, PA, VL, AL, PQ, QA, QL, QG, QP, QV, LA, LG, LP, and LV, said dipeptide sequences N- or C-terminally linked with peptide synthesis methods known in the art. It is preferred that L or Q or both are present in such an AIP sequence, as these amino acids have strong autophagy-inhibiting properties throughout a wide range of cells. Moreover, Q facilitates cellular uptake of L, see also figure 1. In a further preferred embodiment, the invention provides a method of medical treatment for reducing duration, preferably cumulative duration of post-operative complications (POC) in a, preferably human, subject considered or thought considered in need of medical treatment of POC, said treatment comprising providing the vascular system of said subject at least per-operatively with a source of autophagy-inhibiting amino acids, preferably an AIP, selected for at least 50%, preferably for at least 75%, more preferably for

at least 90% most preferably for 100% from the group of amino acids consisting of A, Q, G, V, L and P. Q and/or L are most preferred. In another further preferred embodiment, the invention provides a method of medical treatment for reducing duration, preferably cumulative duration of post-operative complications (POC) in a subject, said treatment

5 comprising providing the vascular system of said subject at least per-operatively with a source of autophagy-inhibiting amino acids, preferably an AIP, selected for at least 50%, preferably for at least 75%, more preferably for at least 90% most preferably for 100% from the group of amino acids consisting of A, Q, G, and V. For example, it was found that when a solution of tetrapeptide AQGV, an autophagy-inhibiting peptide (AIP) comprising a source of

10 autophagy-inhibiting peptides consisting of alanine (A), glutamine (Q), glycine (G) and valine (V), was given intravenously during surgery, neither occurrence nor severity of POC were significantly changed, whereas duration of POC was significantly reduced. In a further preferred embodiment, the invention provides a method of medical treatment for reducing duration, preferably cumulative duration of post-operative complications (POC) in a subject,

15 said treatment comprising providing the vascular system of said subject at least per-operatively with a source of autophagy-inhibiting amino acids, preferably an AIP, selected for at least 50%, preferably for at least 75%, more preferably for at least 90% most preferably for 100% from the group consisting of A, Q, L and P.

In a further preferred embodiment, the invention provides a method of medical treatment

20 for reducing duration, preferably cumulative duration of post-operative complications (POC) in a subject, said treatment comprising providing the vascular system of said subject at least per-operatively with a source of autophagy-inhibiting amino acids, preferably an AIP, wherein said source of autophagy-inhibiting amino acids comprises a dipeptide sequence selected from the AIP group AQ, QQ, LQ, GQ, PQ, VQ, AL, LL, QL, GL, PL, PA, VL, AL, PQ, QA,

25 QL, QG, QP, QV, LA, LG, LP, and LV, or an AIP dipeptide at least functionally equivalent to a dipeptide selected from the group AQ, QQ, LQ, GQ, PQ, VQ, AL, LL, QL, GL, PL, PA, VL, AL, PQ, QA, QL, QG, QP, QV, LA, LG, LP, and LV. Said dipeptide sequence is preferably extended (N- or C-terminally) with at least 1, preferably at least 2 amino acids selected from the from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V),

30 leucine (L), isoleucine (I), proline (P) and arginine (R), to improve (increase) circulation time in the vascular system after intravenous infusion.

In a further preferred embodiment, the invention provides a method of medical treatment for reducing duration, preferably cumulative duration of post-operative complications (POC) in a, preferably human, subject considered or thought considered in need of medical treatment of POC, said treatment comprising providing the vascular system of said subject at least per-operatively with a source of autophagy-inhibiting amino acids, preferably an AIP, wherein said source of autophagy-inhibiting amino acids comprises a tripeptide sequence selected from the AIP group AQG, QQG, LQG, GQG, PQG, VQG, ALG, LLG, QLG, GLG, PLG, VLG, VLA, VLP, PAL, ALP, QAG, QLG, QGG, QPG, QVG, LAG, LGG, LPG, and LVG, or an AIP tripeptide at least functionally equivalent to a tripeptide selected from the group AQG, QQG, LQG, GQG, PQG, VQG, ALG, LLG, QLG, GLG, PLG, VLG, VLA, VLP, PAL, ALP, QAG, QLG, QGG, QPG, QVG, LAG, LGG, LPG, and LVG. Said tripeptide sequence is preferably extended (N- or C-terminally) with at least 1, preferably at least 2 amino acids selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R), to improve (increase) circulation time in the vascular system after intravenous infusion.

In a further preferred embodiment, the invention provides a method of medical treatment for reducing duration, preferably cumulative duration of post-operative complications (POC) in a, preferably human, subject considered or thought considered in need of medical treatment of POC, said treatment comprising providing the vascular system of said subject at least per-operatively with a source of autophagy-inhibiting amino acids, preferably an AIP, wherein said source of autophagy-inhibiting amino acids comprises a tetrapeptide sequence selected from the AIP group AQGV, QQGV, LQGV, GQGV, PQGV, VQGV, ALGV, LLGV, QLGV, GLGV, PLGV, VLGV, QAGV, QLGV, QGGV, QPGV, QVG, LAGV, LGGV, LPGV, and LVGV, or an AIP tetrapeptide at least functionally equivalent to a tetrapeptide selected from the group AQGV, QQGV, LQGV, GQGV, PQGV, VQGV, ALGV, LLGV, QLGV, GLGV, PLGV, VLGV, QAGV, QLGV, QGGV, QPGV, QVG, LAGV, LGGV, LPGV, VLAP, VALP, PALP, VLPA, VLPG and LVGV.

The invention also provides a source of autophagy-inhibiting amino acids selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R), as a medicament for use in a treatment to reduce duration, preferably cumulative duration, of post-operative complications (POCs) in a subject. In a preferred embodiment, such a source as herein provided comprises an

autophagy-inhibiting-peptide (AIP) comprising autophagy-inhibiting amino acids selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R), said AIP a medicament for use in a treatment to reduce duration, preferably cumulative duration, of post-operative

- 5 complications (POCs). In a preferred embodiment, said amino acids in said source or AIP according to the invention are selected for at least 50%, preferably for at least 75%, more preferably for at least 90% most preferably for 100% from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R), for selection as a medicament, such as a solution for intravenous
- 10 administration, for use in a treatment to reduce duration, preferably cumulative duration, of post-operative complications (POCs) in a subject. In another preferred embodiment said source of autophagy-inhibiting amino acids, preferably an AIP, is selected for at least 50%, preferably for at least 75%, more preferably for at least 90% most preferably for 100% from the group consisting of A, Q, G, V, L and P. Q and/or L are most preferred. In another
- 15 preferred embodiment said source of autophagy-inhibiting amino acids, preferably an AIP, is selected for at least 50%, preferably for at least 75%, more preferably for at least 90% most preferably for 100% from the group consisting of A, Q, G, and V. In another preferred embodiment said source of autophagy-inhibiting amino acids, preferably an AIP, is selected for at least 50%, preferably for at least 75%, more preferably for at least 90% most preferably
- 20 for 100% from the group consisting of A, Q, L and P.

- In a further preferred embodiment, the invention provides a source of autophagy-inhibiting amino acids, preferably an AIP, wherein said source of autophagy-inhibiting amino acids at least comprises a dipeptide selected from the group AQ, QQ, LQ, GQ, PQ, VQ, AL, LL, QL, GL, PL, PA, VL, AL, PQ, QA, QL, QG, QP, QV, LA, LG, LP, and LV, or an AIP dipeptide at least
- 25 functionally equivalent to a dipeptide selected from the group AQ, QQ, LQ, GQ, PQ, VQ, AL, LL, QL, GL, PL, PA, VL, AL, PQ, QA, QL, QG, QP, QV, LA, LG, LP, and LV, for use a method of medical treatment for reducing duration, preferably cumulative duration of post-operative complications (POC) in a subject, said treatment comprising providing the vascular system of said subject at least per-operatively with such a dipeptide as provided herein. Preferably said
- 30 dipeptide is provided in a solution for at least per-operative intravenous administration, for

use in a treatment to reduce duration, preferably cumulative duration, of post-operative complications (POCs) in a subject, preferably a human subject.

In a further preferred embodiment, the invention provides a source of autophagy-inhibiting amino acids, preferably an AIP, wherein said source of autophagy-inhibiting amino acids at least comprises a tripeptide selected from the group AQQ, QQG, LQG, GQG, PQG, VQG, ALG, LLG, QLG, GLG, PLG, VLG, VLA, VLP, PAL, ALP, QAG, QLG, QGG, QPG, QVG, LAG, LGG, LPG, and LVG, or an AIP tripeptide at least functionally equivalent to a tripeptide selected from the group AQQ, QQG, LQG, GQG, PQG, VQG, ALG, LLG, QLG, GLG, PLG, VLG, VLA, VLP, PAL, ALP, QAG, QLG, QGG, QPG, QVG, LAG, LGG, LPG, and LVG for use in method of medical treatment for reducing duration, preferably cumulative duration of post-operative complications (POC) in a, preferably human, subject considered or thought considered in need of medical treatment of POC, said treatment comprising providing the vascular system of said subject at least per-operatively with such a tripeptide as provided herein. Preferably said tripeptide is provided in a solution for at least per-operative intravenous administration, for use in a treatment to reduce duration, preferably cumulative duration, of post-operative complications (POCs) in a subject, preferably a human subject.

In a further preferred embodiment, the invention provides a source of autophagy-inhibiting amino acids, preferably an AIP, wherein said source of autophagy-inhibiting amino acids at least comprises a tetrapeptide selected from the group AQQV, QQGV, LQGV, GQGV, PQGV, VQGV, ALGV, LLGV, QLG, GLGV, PLGV, VLG, VLA, VLP, PAL, ALP, QAG, QLG, QGG, QPG, QVG, LAG, LGG, LPG, and LVG, or an AIP tetrapeptide at least functionally equivalent to a tetrapeptide selected from the group AQQV, QQGV, LQGV, GQGV, PQGV, VQGV, ALGV, LLGV, QLG, GLGV, PLGV, VLG, VLA, VLP, PAL, ALP, QAG, QLG, QGG, QPG, QVG, LAG, LGG, LPG, VLA, VALP, PALP, VLPA, VLPG and LVGV for use a method of medical treatment for reducing duration, preferably cumulative duration of post-operative complications (POC) in a, preferably human, subject considered or thought considered in need of medical treatment of POC, said treatment preferably comprising providing the vascular system of said subject at least per-operatively with such a tetrapeptide as provided herein. Preferably said tetrapeptide is provided in a solution for at least per-operative intravenous administration, for use in a treatment to reduce duration, preferably cumulative duration, of post-operative complications (POCs) in a subject, preferably a human subject.

Where for AQ fast hydrolysis within the vascular system is reported (Dipeptiven datasheet) which probably takes place exclusively in the extracellular space, and will rapidly disperse throughout the body, slightly longer peptides, such as LQGV or AQGV, last longer in the vascular environment and will be more easily taken up and processed in cells relevant for its therapeutic effect. Indeed, in lethal animal models of sepsis [41-43], an-order-of-ten-higher weight/volume level of source of amino acids (i.e. the dipeptide versus the tetra- and pentapeptide, respectively) were required for the dipeptide AIP versus the tetra- and pentapeptide AIP carrying that source into the vascular system.

Per-operative intravenous administration of a solution according to the invention may start right at around the begin of surgery, for example at around the time when the patient enters the operating area or receives a first infusion, or even before when the patient receives or is connected to an intravenous infusion device such as a cannula, syringe or a needle, or is connected to an infusion pump set to infuse fluids, medication or nutrients into a patient's vascular (circulatory) system. As the surgery-induced stress response is very similar to the stress response triggered by injuries, it is generally advantageous to start the infusion with a solution comprising a source of autophagy-inhibiting amino acids or peptide (AIP) at around the time of the first surgical knife to skin incision. A graphical description of the hypothalamic activation of the neuroendocrine surgical response at the time of said incision is given in figure 1 of [5].

The invention also provides a method of medical treatment (not nutritional treatment) for reducing cumulative duration of post-operative complications (POC) in a, preferably human, subject considered or thought considered in need of medical treatment of POC, said treatment comprising providing the vascular system of said subject at least per-operatively (during surgery) with a source of autophagy-inhibiting amino acids selected for at least 50%, preferably for at least 75%, more preferably for at least 90% most preferably for 100% from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R), wherein said source of autophagy-inhibiting amino acids is also provided to said subject prior to surgery, preferably wherein said subject is deemed suffering from pre-operative sepsis or pre-operative injuries and requires surgery.

In general, there is no distinct advantage to starting the infusion before a surgical stress response is expected, except when a patient is considered to suffer from pre-operative sepsis or from pre-operative injuries, be it from the battlefield or civilian accidents. Septic patients come to the operating room regularly and they need resuscitation and source control. As reviewed [44], perioperative sepsis is deadly; 40% of cardiac arrests in the perioperative period were associated with sepsis, and these patients had a mortality of 77%. Some of these post-operative deaths might be preventable by treating said patients not only per-operatively (during surgery) but also pre-operatively (before surgery) with a source of autophagy-inhibiting amino acid or peptide (AIP). Such pre-operative treatment followed by per-operative treatment helps reduce the duration of post-operative complications and therewith reduce mortality. Management of severely injured patients set out for operation remains a challenge, as reviewed in [45]. Typically, various types of severe trauma are the ones mostly responsible for the 4.4 million injury-related deaths recorded across the world in 2021 (8% of all deaths). In its different forms (e.g. battlefield or road traffic accidents, homicide, suicide) trauma remains the primary cause of death for those below the age of 45 years. Some of post-operative deaths seen in trauma patients are preventable by treating said patients not only per-operatively (during surgery) but also pre-operatively (before surgery) with a source of autophagy-inhibiting amino acid or peptide (AIP). Such pre-operative treatment followed by per-operative treatment helps reduce the duration of post-operative complications and therewith reduce mortality.

The invention also provides a method of medical treatment (not nutritional treatment) for reducing cumulative duration of post-operative complications (POC) in a, preferably human, subject considered or thought considered in need of medical treatment of POC, said treatment comprising providing the vascular system of said subject at least per-operatively (during surgery) with a source of autophagy-inhibiting amino acids selected for at least 50%, preferably for at least 75%, more preferably for at least 90% most preferably for 100% from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R) wherein said source of autophagy-inhibiting amino acids is also provided to said subject after surgery, preferably wherein said subject is deemed suffering from a systemic inflammatory response after surgery. It is for example preferred to extend or prolong the intravenous treatment procedure with the

autophagy-inhibiting amino acid or peptide solution until after the patient is expected to experience the inflammatory-immune response that often mounts or follows in a second phase of the surgical stress response after the per-operative neuroendocrine-metabolic response so that the patient may also benefit from the beneficial anti-inflammatory effects of said amino acid or peptide solution as well. In certain conditions of surgery, such as in CABG procedures such mounting of an inflammatory-immune response is relatively delayed to a later phase. Then, the anti-inflammatory effects of a solution according to the invention comprising a source of autophagy-inhibiting amino acids for use in per-operative treatment to reduce duration, preferably cumulative duration, of post-operative complications as provided herein may remedy local as well as systemic inflammatory surgical stress responses occurring in that later phase. Preferably such intravenous per-operative treatment may be extended post-operatively, for example to at least 30, preferably to at least 90 minutes after terminating surgery, preferably at least until said patient has been transferred away from the operating room and brought to the ICU, and treatment with said solution may be resumed later as well. Typically, in Coronary Artery Bypass Grafting (CABG) patients treated at least per-operatively with 90mg/kg/hr EA-230 intravenously to reduce duration of POC, it is preferred to extend or prolong the treatment procedure with the autophagy-inhibiting peptide until after the patient has been detached from a cardiopulmonary bypass machine that provided extracorporeal circulation in order to also benefit from additional effects, such as the beneficial anti-inflammatory effects, of said peptide. Preferably such treatment may be extended for at least 30 to 90 minutes after terminating the cardiopulmonary bypass, preferably at least until said patient has been transferred away from the operating room and brought to the ICU.

When desired, degradation of AIP into individual (autophagy-inhibiting) amino acids can be further delayed when said source of autophagy-inhibiting amino acids is additionally provided with a receptor-specific chemotactic target motif that allows to target said autophagy-inhibiting compound to a specific cell carrying said specific receptor. Accordingly, it is preferred that an AIP is additionally provided with a receptor-specific chemotactic target peptide motif, preferably wherein said receptor is selected from the group consisting of elastin-receptor-complex, formyl-peptide-receptor, complement-receptor, and CxC-receptor. A suitable receptor-specific chemotactic target motif may be selected from the group of

peptide motifs (with amino acids in the one-letter-code), xGxxPG, fMLP, WKYVMVm, PGP, AcPGP, SGP, AcSGP, YSFKDMQLGR and AcYSFKPMPLaR that are each individually known in the art to target receptors on the surface of vascular and/or immune cells and can carry an AIP to such cells.

- 5 The invention provides a method for reducing duration of post-operative complications (POC) in a subject considered in need thereof comprising at least per-operatively providing the vascular system of said subject, preferably intravenously, with a source of autophagy-inhibiting amino acids, said source preferably a peptide (herein also identified as an autophagy-inhibiting-peptide (AIP), said amino acids preferably for at least 50%, more
10 preferably for at least 75%, selected from the group of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R). Typically, as the invention herein utilizes a molecular mode-of-action (MoA) of the group of autophagy-inhibiting peptides, such inhibiting effects do not necessarily depend on an exact peptide sequence of an AIP. Instead, their constituent amino acids provide a source of
15 common metabolic, "no-danger or tissue-repair" signals to the nutrient-sensing system of mTOR, leading to physiological inhibition of autophagy and resulting in resolve of disease. These tissue-repair signal molecules change the balance of proteogenesis versus proteolysis in a cell of and may lead to resolve of disease in three steps:

- 1 Administered peptide or amino acid fragments thereof are for taken up by amino acid
20 transport, PEPT1/2 transport, by common endocytosis or by receptor mediated endocytosis or phagocytosis.

2 Internalized peptide is hydrolyzed, and its amino acids are presented to the nutrient-sensing system of mTOR.

- 3 Particular amino acids as identified herein inhibit autophagy more than other amino acids,
25 therewith inhibiting proteolysis and leading to proteogenic resolve and pharmaceutical effect.

The present application provides that such pharmaceutical effect of an AIP in the pre-operative setting is surprisingly evidenced by a significantly reduced duration of POC, and moreover in particular, by a significantly reduced duration of clinically relevant POC, that
30 relate to length-of-stay in hospital and the need to treat such a patient with relevant therapeutic interventions. Typically, the number of POC and severity-scores of POC seem

not significantly changed by AIP-treatment. Not wishing to be bound by theory, one may postulate that pre-operative treatment with AIP (or another source of said autophagy-inhibiting amino acids) does not significantly change the particular risk that a patient has on POC-occurrence, nor that the particular risk on severity of POC per se is changed. However, once a patient experiences a POC, its duration is significantly reduced through AIP-treatment providing said patient with a source of autophagy-inhibiting amino acids. Several findings with the disclosed method and uses of the invention are illustrated in figures 1, 2, 3, 4 and 5 and described in detail below, see also tables 1 and 2. POCs can be identified in various ways known in the art, in a preferred embodiment, the invention provides a method wherein said POCs are identifiable through methods and means (guidance) provided by the Society of Anaesthesiology (ESA) and the European Society of Intensive Care Medicine (ESICM) joint taskforce on perioperative outcome measures and published as Standards for definitions and use of outcome measures for clinical effectiveness research in perioperative medicines, herein also identified as European Perioperative Clinical Outcome (EPCO) definitions. In a further preferred embodiment, said POCs comprise at least 3, preferably at least 6, more preferably at least 9, more preferably at least 12, more preferably at least 15, more preferably at least 18, more preferably at least 21, most preferably at least all 22 single-organ-outcome-measures (SOOMs) as provided in Table 1, first column.

In another preferred embodiment, the invention provides a method for reducing duration of moderate and severe post-operative complications (POCs) in a subject considered in need thereof comprising providing the vascular system of said subject with a source of amino acids, said amino acids for least 50% selected from the group of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R). Where moderate and severe POCs usually require clinical treatment while mild POCs usually do not require clinical treatment, a significant reduction of duration of moderate and severe POC reflects a significant beneficial reduction of clinical severity to the patient as well as need for resources to be supplied by the caring entity to the patient.

Description of Figures

Figure 1. Treatment with AIPs such as EA-230 shift vascular cell activities towards improved tissue repair.

Left: mTOR is a central cell growth regulator, activated by growth factors such as insulin, nutrient and energy levels, and by amino acids. mTOR (complex 1 and 2) connects cellular metabolism and growth with a wide range of environmental inputs and regulates many fundamental cell processes, from synthesis (proteogenesis) to degradation (autophagy) [46]. In general, activation of mTOR (leading to proteogenesis) in cells is upregulated during tissue repair [47, 48], whereas autophagy (leading to proteolysis) increases vascular permeability [49, 50] as well as inflammation [51-53]. mTOR activating amino acids glutamine as well as leucine are actively imported [54] in cells and act in concert to stimulate proteogenesis and inhibit autophagy, shifting the cells' balance from vascular permeability and inflammation towards tissue repair with cell survival and growth. Right: AIPs such as EA-230 add further mTOR-activating and autophagy-inhibiting amino acids by common endocytosis and phagocytosis to the cell and further shifts the cell's balance to tissue repair.

Figure 2. Boxplot of duration of moderate and severe (= treatment dependent) SOOMs by treatment group illustrating that per-operative treatment with AIP (here EA-230 (active) is shown) allows significant reduction of duration of POC in comparison to placebo treatment.

Boxplot of duration of moderate and severe SOOMs (in hrs.) by treatment groups placebo (n=30, blue box) and EA-230 (n=30, red box). Boxed areas identify the interquartile range (IQR) from the 25th to the 75th percentile, with the median located within. The median POC-duration was significantly attenuated by 27.1 hrs from 44.3 hrs in the placebo group to 17.2 hrs in EA-230 (p=0.0026). With placebo and EA-230, four (dots and star) respectively 1 (dot) observation(s) of SOOM-duration are larger than a maximum defined by (Q3 + 1.5*IQR), illustrating a broader spread with a significant tendency towards toward longer duration of SOOMs in the placebo group versus EA-230. The boxplot shows the overall SOOM response pattern in patients without (placebo, n=30) or with active (EA-230, n=30) treatment. The post-hoc POCs analysis of the EASI study showed that duration of moderate of severe (treatment dependent) POC was significantly reduced by 61% in patients administered EA-230 (n=30) compared to placebo (n=30) (median POC-duration 17.2 hr [9.4 – 32.0] vs

44.3 hr [10.1-171.0]; $p=0.0026$). Thus, taking all found SOOMS together, placebo patients required 2.5 times longer duration of treatment-dependent Post-Operative Complications (POCs; $P=0.0026$) and demonstrated 25% longer Length-of-Stay in the hospital (LoS; $P=0.001$) than EA-230 patients. These findings translate into an average POC duration of
 5 ~0.5 days in EA-230 treated patients versus ~2.8 days in placebo treated patients. Thus, the double-blinded post-hoc POCs analysis confirmed a reduction in POC-duration of approximately 2 days, relating well to the observed reduction in hospital-LoS (8 days [7-11] vs 10 days [8-12]). To further illustrate the treatment effects of EA-230 beyond its significant effects on the duration of POC requiring clinical treatment, figure 3 illustrates
 10 that shorter POC-duration with EA-230 is particularly evident in the ~ first 100 hrs after operation whereas placebo patients typically show longer SOOM duration and simultaneously generally showed a higher fluid balance during days 1-3.

Figure 3. Scatter plot of individual moderate and severe (= treatment dependent) SOOM
 15 observations of defined POC duration illustrating that per-operative treatment with AIP (here EA-230 (active) is shown) significantly reduces the net-fluid-balance of patients in comparison to placebo treatment.

A scatter plot of individual moderate and severe SOOM observations of defined duration (y-axis, SOOM-hrs) against the individual cumulative NFB calculated over post-operative days
 20 0 - 3 (x-axis). Y-axis duration of moderate/severe SOOMs (in hrs) by treatment groups placebo (n=30, blue) EA-230 (n=30, red) set out against the cumulative NFB (in mL) from post-operative days 0 - 3. Placebo treated patients demonstrate a peri-operative exacerbated net fluid balance (NFB) (e.g., fluid overload) with increased duration of moderate and severe POCs, whereas EA-230 treated patients demonstrate less fluid
 25 overload related to their reduced duration of moderate and severe POCs.

Figure 4.

Scatter plot of individual moderate and severe (= treatment dependent) SOOM observations of defined POC duration illustrating that per-operative treatment with AIP (here EA-230 (active) is shown) significantly reduces the time it takes from first incision at which
 30 treatment with IMP was initiated up to the time the last SOOM occurrence was detected

and also significantly allows reduction of duration of POC in comparison to placebo treatment.

A scatter plot of individual moderate and severe SOOM observations of defined duration (y-axis, SOOM-hrs) against the time range (x-axis, Hrs_IMP_SOOM) wherein SOOMs arise after the provision of treatment (EA-230 n= 30, placebo n=30). Of note: 2 placebo observations are not shown, each occurring more than 400 hrs. after provision of EA-230. Shorter POC-duration with EA-230 is particularly evident in the first 100 hrs. after operation, again illustrating that EA-230 renders the patient with a beneficial recovery status over placebo.

Figure 5. Scatter plot of individual mild, moderate and severe (= treatment dependent) SOOM observations of defined POC duration divided in placebo (top) and active (bottom) treatment. Patients were treated with the investigative medical product (IMP) for as long as the operation lasted, as defined above. Where the trendline in the placebo treatment shows an upward slant, typically corroborating established observations in the art that the longer the operation lasts, the longer the patient stays in the hospital, the trendline in the active group treated with AIP shows a downward slant, demonstrating that EA-230 renders the patient with a beneficial recovery status over placebo and that longer treatment typically increases that benefit.

Top: Length-of-stay in the hospital (days) of patients treated with placebo (y-axis) is plotted against the duration of treatment with the IMP in minutes (x-axis). In particular, the prolonged operative time that is found [13] is now herein associated with an increase in the risk of vascular complications and tissue damage that lead to the here increased duration of POC and increased length-of-stay (LOS) in the hospital. As indeed can be observed from the trendline plotted along the with scatter plots, actually prolonging operation time prolongs LOS. Following that rising trendline, table 4 has calculated an ~ 0.5 day increase in LOS per every half hour extension of operating time.

Bottom: Length-of-stay in the hospital (days) of patients treated with AIP (EA-230, y-axis) is plotted against the duration of treatment with the IMP in minutes (x-axis). Treatment with said AIP reverses the rising of the trendline into a lowering, therewith reflecting an actual shortening of the length of the stay in the hospital due to treatment with AIP. As can be seen

in Table 4, an~ 0.3 day decrease in in LOS per every half hour extension of operating time is calculated. These findings translate into a gain of at around 0.8 day decrease in LOS per every half hour treatment with AIP versus placebo, in particular after the patient has received treatment already 1 hour, up to at around 4 to 5 hours of treatment.

5 Detailed description

Following major surgery, patients show a surgical stress response with increased capillary leakage and hemodynamic instability as clinically relevant consequences. Alanyl-Glutamyl-Glycyl-Valine (AQGV), or EA-230, is a small peptide in development for peri-operative application to prevent post-operative clinical complications (POCs) after major surgery.

- 10 Preventive therapy with EA-230 reduces capillary leakage and reduces the need for fluids resulting in less tissue injury and increased tissue repair, with significantly reduced duration of POCs in multiple organs and a likewise significantly reduced Length of Stay (LoS) in the ICU and hospital.

- The on-pump Coronary Artery Bypass Grafting (CABG) procedure is a major surgical
15 procedure typically compromising hemodynamic stability of the patient. To ameliorate peri-operative hemodynamic instability, guidelines for standard of care (SoC) in cardiac surgery recommend fluid therapy and vasopressor/inotropic therapy to improve cardiac preload and tissue perfusion (Wahba et al. 2019; Engelman et al. 2019). Such SoC regimens are established on a per-patient basis governed by the patient's actual needs determined
20 through routine monitoring of the patient. However, these SoC-regimens may paradoxically also add to the causes of POCs by affecting hemodynamic stability in multiple organ systems.

- EA-230's therapeutic properties were reflected in the Phase 2b (EASI)-study which demonstrated a reduction of Net Fluid Balance (NFB), inotropic score, and LoS in ICU and hospital when EA-230 was given during surgery while SoC is provided. EA-230's effect on
25 capillary leakage and POCs must be assessed in multiple organs because capillary leakage occurs systemically and affects all organs. The European Peri-operative Clinical Outcome (EPCO) definitions for organ dysfunction (Jammer et al. 2015) are chosen as clinical measure. Double-blinded re-analysis of the (Serious) Adverse Events ((S)AEs) in the Phase 2b (EASI) study according to the EPCO definitions confirmed the positive effect of EA-230 on duration
30 of POCs.

Autophagy-inhibiting peptides.

One letter code

In describing protein or peptide composition, structure, and function herein, reference is made to amino acids. In the present specification, amino acid residues are identified by using the following abbreviations. Also, unless explicitly otherwise indicated, the amino acid sequences of peptides and proteins are identified from N-terminal to C-terminal, left terminal to right terminal, the N-terminal being identified as a first residue. Ala: alanine residue; Asp: aspartate residue; Glu: glutamate residue; Phe: phenylalanine residue; Gly: glycine residue; His: histidine residue; Ile: isoleucine residue; Lys: lysine residue; Leu: leucine residue; Met: methionine residue; Asn: asparagine residue; Pro: proline residue; Gln: glutamine residue; Arg: arginine residue; Ser: serine residue; Thr: threonine residue; Val: valine residue; Trp: tryptophane residue; Tyr: tyrosine residue; Cys: cysteine residue. The amino acids may also be referred to by their conventional one-letter code abbreviations; A=Ala; T=Thr; V=Val; C=Cys; L=Leu; Y=Tyr; I=Ile; N=Asn; P=Pro; Q=Gln; F=Phe; D=Asp; W=Trp; E=Glu; M=Met; K=Lys; G=Gly; R=Arg; S=Ser; and H=His.

Peptides

Peptide shall mean herein a natural biological or artificially manufactured (synthetic) short chain of amino acid monomers linked by peptide (amide) bonds. Glutamine peptide shall mean herein a natural biological or artificially manufactured (synthetic) short chain of amino acid monomers linked by peptide (amide) bonds wherein one of said amino acid monomers is a glutamine. Chemically synthesized peptides generally have free N- and C-termini. N-terminal acetylation and C-terminal amidation reduce the overall charge of a peptide; therefore, its overall solubility might decrease. However, the stability of the peptide could also be increased because the terminal acetylation/amidation generates a closer mimic of the native protein. These modifications might increase the biological activity of a peptide and are herein also provided.

Peptide synthesis

In this application, peptides are either synthesized by classically known chemical synthesis on a solid support (Ansynth BV, Roosendaal, The Netherlands) or in solution (Syncom BV, Groningen, The Netherlands and Diosynth BV, Oss, The Netherlands). Pharmaceutical

peptide compositions may be synthesized using trifluoroacetate as a counter-ion or salt after which trifluoroacetate is exchanged by a counter-ion such as maleate (from maleic acid), acetate (from acetic acid), tartrate (from tartaric acid) or citrate (from citric acid). The drug substance of AQGV (EA-230) for use in pre-clinical and clinical human studies has been manufactured by Organon N.V (formerly Diosynth B.V.), (Oss, The Netherlands), whereas filling and finishing of the final product has been performed by Octoplus Development, Leiden (The Netherlands). Molecular weight of EA-230 (AQGV) is 373g/mol).

Determination of AIP chemotactic activity and functional equivalence.

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Blood is drawn from healthy volunteers into tubes containing citrate as an anticoagulant. Neutrophils are isolated by using a Polymorphprep kit (Nicomed, Oslo, Norway) according to the manufacturer's instructions; monocytes are purified with magnetic beads (Miltenyi Biotech). The purity of the cells, as assessed by flow cytometry (anti-CD45, 14, DR, and CD66b), is > 93%. For each cell type, samples from two different donors are examined. Alternatively, human U937 monocytic cells are purchased from the American Type Culture Collection (ATCC catalog number CRL-1593.2, Manassas, Va). Cells are maintained in suspension culture in T-75 flasks containing RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics, and cultures are split every 3 to 5 days. Three days before use in chemotaxis assays, U937 cells are stimulated to differentiate along the macrophage lineage by exposure to 1 mmol/L dibutyryl cyclic adenosine monophosphate (dbcAMP; Sigma Chemical Co), as described. Cells are washed three times to remove culture medium and then resuspended in chemotaxis medium (Dulbecco's modified essential medium supplemented with 1% lactalbumin hydrolysate) for plating into assay chambers at a final concentration of 2.5×10^6 cells/mL. Chemotaxis assays are performed in 48-well microchemotaxis chambers (Neuro Probe, Cabin John, Md). The bottom wells of the chamber are filled with 25 mL of the AIP chemotactic stimulus (or medium alone) in triplicate. An uncoated 10-mm-thick polyvinylpyrrolidone-free polycarbonate filter with a pore size of 5 mm is placed over the samples (Neuro Probe). The silicon gasket and the upper pieces of the chamber are applied, and 50 mL of the monocyte cell suspension are placed into the upper wells. Chambers are incubated in a humidified 5% CO₂ atmosphere for 3 hours at 37° C, and nonmigrated cells are gently wiped away from the upper surface of

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the filter. The filter is immersed for 30 seconds in a methanol-based fixative and stained with a modified Wright-Giemsa technique (Protocol Hema 3 stain set; Biochemical Sciences, Inc, Swedesboro, NJ) and then mounted on a glass slide. Cells that are completely migrated through the filter are counted under light microscopy, with 3 random high-power fields (HPF; original magnification x 400) counted per well. Human monocytes are isolated from freshly drawn blood of healthy volunteers using serial Ficol I/Pe lasti n receptor complex (ERC)oll gradient centrifugation, as described elsewhere. Cells are cultured for 16 hours in RPMI-1640 media supplemented with 0.5% human serum to become quiescent after isolation. Purity of the cells is >95% as determined by flow cytometry analysis. Monocyte chemotaxis is assayed in a 48-well microchemotaxis chamber (Neuroprobe, Gaithersburg, MD) in serum-free media. Wells in the upper and lower chamber are separated by a polyvinylpyrrolidone-free polycarbonate membrane (pore size 5 µm; Costar). Freshly isolated monocytes at a density of 5×10^5 /mL are incubated for 2.5 hours with recombinant C-peptide (Sigma), before migrated cells on the bottom face of the filter are stained and counted under the light microscope. Maximal chemotactic activity is measured with 0.1 mmol/L N -formyl-methionyl-leucyl-phenylalanine (f-MLF; Sigma Chemical Co), and checkerboard analysis is used to distinguish chemotaxis from chemokinesis. Likewise, additional determination of functionally equivalent AIP activity is undertaken in immune cells such as monocytes or monocytic cells for example after FPR-, p38-, or otherwise kinase activation as discussed in WO2022069576. FPR-ligand fMLP causes rapidly induced changes in phosphorylation status of PKB (also known as AKT) and p38 MAPK kinases. AIP-peptide effects on p38 MAPK are for example detected after FPR- stimulation, AIP-peptide effects on PKB(AKT) may also be studied. AIP effects on p38 and PKB-mediated signalling shows specific and rapid effects of autophagy-inhibiting-peptide on p38 signaling in the context of regulation of the PI3K/AKT/mTOR pathway.

Major surgery [55] has been associated with undesirable sequelae such as pain, cardiopulmonary, infectious, and thromboembolic complications, cerebral dysfunction, nausea and gastrointestinal paralysis, fatigue, and prolonged convalescence for many years already. Development of new strategies to improve post-operative outcome is acknowledged as an unmet medical need [56]. The key pathogenic factor in post-operative morbidity and resulting mortality is the surgical stress response with reduced exposure of

- tissues to nutrients and oxygen, often with microvascular ischemia and subsequent increased demands on organ function. The surgical stress response is typically further enhanced in surgery demanding cardioplegic arrest such as CABG [57] that lead to enhanced ischemia reperfusion injury (IRI). As a consequence, Danger Associated Molecular Patterns (DAMPs) are released from cells and Pathogen Associated Molecular Patterns (PAMPs) translocate from the intestines. Changes in organ function are thought to be mediated by IRI-induced activation of the PAMP/DAMP signaling pathways [58-61], e.g., higher plasma levels of DAMPs correlated with a significantly longer need for post-operative vasopressor support [62].
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- 10 DAMPs bind pattern recognition receptors (PRR) such as Toll-like receptors (TLRs) and formyl peptide receptors on non-immune cells (endothelial cells and vascular smooth muscle cells) and trigger cytoskeletal rearrangement with myogenic vascular contraction ending in endothelial barrier dysfunction and increased expression of leucocyte adhesion factors [63, 64]. This, in turn, promotes capillary leakage with leukocyte extravasation and
- 15 disturbed tissue perfusion [65, 66].

Hemodynamic instability results in inadequate tissue perfusion and oxygenation [25, 26]. To address these conditions, patients are treated with fluids (to increase cardiac preload) and inotropics (to increase cardiac output) and/or vasopressors (to increase vascular tone). While this may improve the acute consequences of hemodynamic instability, both fluid

20 overload as well as an increased need for hemodynamic support using inotropics and/or vasopressors (reflected in a higher inotropic score) are associated with increased POCs and increased LoS in the ICU and hospital, and are important predictors of both short-term and long-term mortality [28-31].

IRI primarily occurs locally, but its mediators can infiltrate the systemic circulation and

25 influence other remote non-ischemic organ systems [26]. These pathophysiological processes may culminate, in some tissue areas, in a 'no-reflow/reflow paradox' [67], whereby reperfusion (reflow) after transient tissue ischemia (no-reflow) paradoxically aggravates ischemic tissue conditions and constitutes a further need to improve tissue viability. PAMPs/DAMPs mediate such heterogenous IRI effects by preparing the endothelial

surface for leukocyte infiltration with increased expression of leukocyte-adhesion molecules such as vCAM-1 and E-selectin [68-70], thereby driving disease progression [71].

PAMP/DAMP signaling also contributes to Disseminated Intravascular Coagulation (DIC) that can lead to both microvascular and macrovascular clotting and compromised blood flow [72] with multiple organ dysfunction syndrome as a result [73]. Typically, DIC is of a heterogenous nature, affecting some tissue areas whereas other tissue areas may not be impacted; DIC thus also contributes to the heterogenous nature of POCs, and can be an explanation for the diverse nature of POCs found in patients recovering from surgery.

The central modulator mammalian-target-of-rapamycin (mTOR), a hypoxia and nutrient sensor and master regulator of cellular metabolism [74] is herein considered to be involved in the mechanism of action of autophagy-inhibiting peptides (see also figure 1). As expected for small peptides [75], the prototype AIP tetrapeptide L-Alanyl-L-Glutamyl-Glycyl-L-Valine (EA-230) has a short half-life *in vivo*, and rapidly disintegrates into its individual amino acid constituents alanine (A), glutamine (Q), valine (V) and glycine (G). Functional equivalents of AQGV are for example LQGV or LAGV, and other peptides with amino acids that activate mTOR and conversely inhibit autophagy. mTOR is classically activated by amino acids [46], some of which (leucine, (L), proline (P), isoleucine (I) arginine (R) and EA-230's constituent amino acids A, Q, G and V) are known to activate mTOR (and inhibit autophagy) more than others [76-78]. The amino acid glutamine (Q) is the most important mTOR signaling molecule derived from EA-230 and can be interchanged with leucine (L). Both leucine and glutamine are particularly strong inhibitors of cellular autophagy and activator of mTOR [79, 80] and glutamine has been shown to reduce tissue injury [81, 82], even when used alone. Activities improve when presented within the context of short glutamine-containing peptides such as dipeptide AQ, LQ or GQ [83, 84]. Glutamine may further positively regulate the mTOR pathway by facilitating the uptake of mTOR activator leucine [54] and by promoting mTOR assembly and lysosomal localization [85, 86]. The fact that the tetrapeptide EA-230 is rapidly disintegrating into amino acids that are used in common metabolic processes of the cell may well contribute to the inherent beneficial safety profile reported for EA-230.

Clinical effectiveness trials should be designed to test whether peri-operative interventions can improve or prevent POCs and therefore be cost-effective and relevant to patients.

Vascular complications, with capillary leakage and decreased tissue perfusion after major surgery, such as CABG, affect multiple organ systems and lead to POCs of a widely diverse

5 nature, e.g., in the brain it can appear as delirium, in the lungs as a longer need for mechanical ventilation, in the cardiovascular system as a need for inotropes / vasopressors, in the cardiac system as atrial fibrillation, and in the kidneys as acute kidney injury (AKI). Due to this diverse nature of the complications and the inter-individual variability in the organs affected, a standardized approach that allows for objective assessment of post-
10 operative clinical outcome in multiple organs is needed to assess and objectively compare therapeutic interventions. Clinical outcome measures used in such trials must be robust, clearly defined, and patient relevant. The frequency of post-operative complications is used as an indicator of surgical quality; however, comparison of outcomes may be hampered by a lack of agreement on the definition of complications and their severity. Common disease
15 severity measures like Acute Physiology and Chronic Health Evaluation (APACHE) and Sequential Organ Failure Assessment (SOFA) score were generally not deemed fully suitable to monitor POCs because the monitoring is too short (APACHE only covers the first post-operative 24 hrs) or only rates severe organ failure (SOFA score). More standardized patient-centered outcomes that suit the purpose have however been provided recently.

20 The Society of Anaesthesiology (ESA) and the European Society of Intensive Care Medicine (ESICM) joint taskforce on perioperative outcome measures published the Standards for definitions and use of outcome measures for clinical effectiveness research in perioperative medicines (European Perioperative Clinical Outcome (EPCO) definitions [87]. For adverse events, 30 days is recommended as the most appropriate follow-up period, although some
25 composite outcomes may be reported on a prespecified post-operative day. However, mortality at 30 days does not provide an adequate measure of the clinical effectiveness of a treatment designed to improve patient-centered outcomes. Many patients who develop severe complications may survive up to 30 days only to die in the weeks that follow. In addition, the event rate for 30-day mortality may be too low, creating methodological
30 problems in terms of sample size. The EPCO group agreed that most patients undergo surgery in the hope of long-term survival with a good functional outcome, and therefore

recommended that all trials should report mortality at a minimum of 90 days after surgery and ideally 1 year, although short-term mortality may remain relevant as a treatment safety outcome. The EPCO-based clinical effectiveness standard defines 22 individual Single Organ Outcome Measures (SOOMs), identifiable as POCs (see Table 1), with a system of severity-score grading defining “Mild”, “Moderate” and “Severe” for each SOOM. The EPCO definitions have been proposed as robust and clearly defined clinical outcome measures for the identification of POCs. Clinical effectiveness may be assessed by registration of the complications that are identified as SOOM according to the EPCO definitions. Emphasis may be given to moderate and severe SOOMs since these are considered most-relevant to patients, since the more serious SOOMs usually require clinical treatment whereas SOOMs defined as mild result in only temporary harm and would not usually require specific clinical treatment other than standard of care. Rating into “Moderate” and/or “Severe” according to EPCO criteria generally reflects grading from Grade II onwards in the Clavien-Dindo grading of POC [88], where rating into “Mild” in EPCO corresponds to Grade I in Clavien-Dindo. The EPCO grading system is considered suitable for very pragmatic trials, in particular international clinical trials. Several other grading systems were assessed and the Clavien-Dindo grading system is considered a widely used and valid alternative for EPCO [87]. The Clavien-Dindo criteria, albeit widely used to assess POC-state per patient, do not or only little identify individual POC and restrict severity grading to overall patient condition mainly. Thus, following EPCO criteria was deemed by us useful to obtain sufficiently detailed information on the AE data set used and we used the EPCO criteria in a double-blinded post-hoc analysis of the EASI study AE-data set in the figures discussed below, as Clavien-Dindo scoring can be derived from EPCO-scoring, but not vice versa.

A post-hoc EPCO-based analysis of POCs in the EASI study is reported here to explore the possibility that EA-230 improves clinical outcome after major surgery by reducing the number of POCs. This analysis on the per-protocol population data of the EASI study was performed to assess the differences between EA-230- and placebo-treated patients in the number of POCs. Due to the diverse and heterogenous nature of said complications and the inter-individual variability in the organs affected, a standardized approach that allowed for objective assessment of post-operative clinical outcome in multiple organs was needed to

assess and objectively compare the effect of therapeutic interventions. The standards for definitions and use of single-organ-outcome-measures (SOOMS) for assessing clinical effectiveness research in perioperative medicines as published by the European EPCO [87] were proposed as robust, clearly defined and patient relevant clinical outcome measure to assess the clinical effect of EA-230 on POCs. It should be emphasized that EPCO definitions should not be considered surrogate markers or composite outcome measure (requiring multiplicity) but rather an approach with one set of definitions that allows to measure POCs of a diverse nature that can show up in multiple organs.

Independent medical experts performed a double-blinded adjudication on the AE-data set of the EASI study per-protocol population to identify the number of subjects with one or more POCs that fulfilled the EPCO definition of SOOMs and to grade the severity of each of these SOOMs. The randomized, double-blinded, placebo-controlled EASI study [37, 38] was conducted in a per-protocol population of 179 patients. Patients older than 18 years and scheduled for elective CABG, with or without valve surgery, with use of cardio-pulmonary bypass (CPB) were eligible for participation. Patients were randomized in a 1:1 ratio to receive either EA-230 (90 mg/kg/hr from the moment of first incision throughout the surgical procedure to stop of CPB, n=90) or placebo (sodium chloride with identical osmolality, appearance, and texture as the EA-230 solution, n=89) at the same administration rate. A total of 500 AEs were reported, 217 AEs in the EA 230 group and 283 AEs in the placebo group. Said AE-data set was found to be not normally distributed. No significant differences were found between the number of AEs in the placebo group when analyzed in the Mann-Whitney test.

In order to prevent bias in the assessment in the adjudication procedures, the defined and accepted EPCO outcome measures were strictly followed, and no differences were made in the adjudication between disease effects, surgery outcomes and effect of EA-230. All SOOMs were deemed equally relevant and no subset of SOOMs or weighing of specific SOOMs was applied because POCs are of diverse nature, and it cannot be judged on beforehand which complications are most relevant. A subsequent statistical analysis (Mann-Whitney) was performed to investigate the effect of EA-230 on the number of POCs as compared to placebo based on the SOOMs identified by applying EPCO-criteria.

The double-blinded adjudication led to a population of 110 patients with 175 SOOMs: 53 patients with 81 SOOMs in the EA-230 arm, and 57 patients with 94 SOOMs in the placebo arm. There is a wide distribution of SOOMs observed which justifies that all SOOMs may be relevant in the target population, see also Table 1, second column. The total number of SOOMs was lower in the EA-230 arm than in the placebo arm in this analysis population. A non-significant reduction of 14% in EA-230 treated patients versus placebo in the total SOOMs was observed.

Due to incomplete time-keeping, from the total group of 110 patients with a SOOM, the SOOM duration (calculated from start and end date and time) could be established from the AE-data set for 71 patients only, with 98 SOOMS: 32 patients with 42 SOOMs in the EA-230 arm and 39 patients with 56 SOOMs in the placebo arm, see also Table 1, third column. Again, the total number of SOOMs was lower in the EA-230 arm than in the placebo arm in this analysis population. An again non-significant reduction of 25% in EA-230 treated patients versus placebo in SOOMs with defined duration was observed.

The final analysis population was determined on the bases of severity grading and considered only those SOOMs of defined duration with grading "Moderate" or "Severe" according to EPCO definitions (Jammer et al 2015). Moderate and severe SOOMs are considered clinical- and patient-relevant, as these reflect the requirement for specific clinical treatment whereas SOOMs classified as "Mild" by EPCO do "not usually require specific clinical treatment".

This third analysis comprises 60 patients with 81 moderate and severe SOOMs with defined duration (Table 1, last column); 30 patients with 37 SOOMs in the EA-230 arm and 30 patients with 44 SOOMs in the placebo arm, see also Table 1, fourth column.

Again, the total number of SOOMs was lower in the EA-230 arm than in the placebo arm in this analysis populations. A non-significant reduction of 16% in EA-230 treated patients versus placebo in the moderate and severe SOOMs with defined duration population, respectively, was observed.

Table 1: POC double-blinded adjudication results Frequency of SOOM occurrences identified in the EASI-study results: Total number of SOOMs, Number of SOOMs with defined duration and Number of moderate and severe SOOMs with defined duration.

	Total SOOMs		SOOMs with defined duration		Moderate and Severe SOOMs with defined duration	
	110 patients		71 patients		60 patients	
	175 SOOMs		98 SOOMs		81 SOOMs	
SOOM classification according to EPCO (Jammer et al 2015)	placebo	EA-230	placebo	EA-230	placebo	EA-230
1.1 Acute Kidney Injury	4	1	2	0	1	0
1.2 Acute Respiratory Distress Syndrome	0	0	0	0	0	0
1.3 Anastomotic breakdown	0	0	0	0	0	0
1.4 Arrhythmia	45	41	28	27	25	25
1.5 Cardiac arrest	0	1	0	1	0	0
1.6 Cardiogenic pulmonary oedema	1	0	1	0	1	0
1.7 Deep vein thrombosis	0	0	0	0	0	0
1.8 Delirium	13	6	9	5	7	4
1.9 Gastrointestinal bleed	0	0	0	0	0	0
1.10 Infection, source uncertain	9	11	8	3	3	2
1.11 Laboratory confirmed bloodstream infection	1	0	1	0	1	0

	Total SOOMs		SOOMs with defined duration		Moderate and Severe SOOMs with defined duration	
	110 patients		71 patients		60 patients	
	175 SOOMs		98 SOOMs		81 SOOMs	
SOOM classification according to EPCO (Jammer et al 2015)	placebo	EA-230	placebo	EA-230	placebo	EA-230
1.12 Myocardial infarction	2	3	2	2	2	2
1.13 Myocardial injury after non-cardiac surgery	0	0	0	0	0	0
1.14 Pneumonia	5	3	0	0	0	0
1.15 Paralytic ileus	1	1	1	1	1	1
1.16 Post-operative haemorrhage	0	3	0	2	0	2
1.17 Pulmonary embolism	0	0	0	0	0	0
1.18 Stroke	0	1	0	0	0	0
1.19 Surgical site infection (superficial)	6	6	3	1	3	1
1.20 Surgical site infection (deep)	2	2	1	0	0	0
1.21 Surgical site infection (organ/space)	0	0	0	0	0	0
1.22 Urinary tract infection	5	2	0	0	0	0
Total POC (= total SOOMs per treatment group)	94	81	56	42	44	37
Total patients	57	49	39	32	30	30

In order to confirm the approach, a further post-hoc analysis of POCs in the EASI study was performed based on the AE dataset consisting of an adjudication of the AE dataset to determine duration, preferably cumulative duration, of POCs and statistical analysis of adjudication results.

- 5 Emphasis was given to the SOOMs with defined duration and with a severity grading of “Moderate” or “Severe” according to EPCO definitions. Moderate and Severe (= Treatment Dependent) SOOMs are considered clinically and patient-relevant, as these reflect the requirement for specific clinical treatment whereas SOOMs classified as “Mild” by EPCO do “not usually require specific clinical treatment” (Jammer et al 2015).
- 10 Contrary to the numbers of SOOMs or POCs, the cumulated duration of all 22 SOOMs per patient (cumulated SOOM duration = total POC duration) was significantly reduced in the EA-230 compared to the placebo group. As the duration data were found non-parametric in nature (Shapiro-Wilks test; $p < 0.05$), the median is a more appropriate measure than the mean, hence the median POC duration was applied for statistical analysis. The median total
- 15 POC-duration was significantly reduced from 42.9 hrs in the placebo arm to 24.1 hrs in the EA-230 arm ($p = 0.0040$, Table -middle column) in the population with a SOOM of defined duration. For the population with moderate or severe SOOMs of defined duration, the median total POC-duration was significantly reduced from 44.3 hrs in the placebo group to 17.1 hrs in EA-230 ($p = 0.0026$, Table -righthand column).

Table 2: POC duration (hrs) for the population with SOOMs of defined duration and for the population with moderate/severe SOOMs of defined duration.

	SOOMs with defined duration		Moderate and Severe SOOMs with defined duration	
	71 patients		60 patients	
	98 SOOMs		81 SOOMs	
Significance determined by Mann-Whitney U test	p=0.0040		p=0.0026	
Treatment group	placebo	EA-230	placebo	EA-230
Mean duration (hrs)	122.8	25.2	119.2	22.6
Standard deviation	199.8	24.1	180.0	18.9
Median duration (hrs)	42.9	17.5	44.3	17.2
Lower quartile	10.0	9.4	10.1	9.4
Upper quartile	157.2	39.3	171.0	32.0
Total POC	56	42	44	37
Total patients	39	32	30	30

Surprisingly, where numbers nor severity-scores of POCs were significantly changed by treatment with EA-230, analyses of the duration of POCs identifiable by EPCO-criteria, here through a double-blinded post-hoc analysis of the EASI study AE-data set, showed that duration of POC, notably also duration of moderate of severe (and clinically relevant) POC was strongly and significantly reduced. As shown in Table 2, duration of moderate and severe POC was by 61% reduced in patients administered EA-230 (n=30) compared to placebo (n=30) (median POC-duration 17.2 hr [9.4 – 32.0] vs 44.3 hr [10.1-171.0]; p=0.0026). These findings (when taking into account also the patients that did not experience POC) translate into an overall average POC duration of ~0.5 days in EA-230 treated patients versus ~2.8 days in placebo treated patients. Thus, the double-blinded post-hoc POCs analysis confirmed a reduction in POC-duration of approximately 2 days, relating well to the

observed reduction in hospital-LoS by treatment with EA-230 (8 days [7-11] vs 10 days [8-12]) as reported earlier for the EASI-study. Of note: when calculating the average cumulated duration of moderate or severe infections per patient (available were data from moderate or severe SOOMs 1.10, 1.11, and 1.19 of defined duration) taken together it was found that the duration of these infections was significantly reduced in EA-230 treated patients versus placebo treated patients (2.3 hours versus 32.6 hours, respectively; P = 0.0304).

To provide further rule-of-thumb guidance to the clinician, gain analyses were undertaken for treatment up to 2.5 hours (150 minutes) compared to prolonged treatment for up to 4 hours (240 minutes). The former showed a gain of 4.8 hours reduction in POC duration, the latter a gain of 7.6 hours, indicating that per-operative treatment with EA-230 strongly benefits from prolonging treatment with EA-230 from 2.5 hours to at least 4 hours after the first incision that indicates the start of surgery.

Table 3 below shows the average POC duration in relation to length-of-stay in ICU (LOS-ICU) and length-of-stay in hospital (LOS-Hos), calculated for the population with lower length POC-duration than median (3 A, n = 37, of which 21 placebo and 16 EA-230) versus the population with longer length POC-duration than median (3 B, n = 33, of which 17 placebo and 16 EA-230) as calculated with 71 patients experiencing mild, moderate and severe SOOMs of defined duration. Gains all show significant better duration outcomes as tested between placebo and EA-230 (T-Test, * indicates p <0.05) when treatment is longer. Typically, shorter POC-duration with EA-230 with improved LOS-ICI and LOS-Hos is particularly evident in the first 100 hrs. after operation, showing a distinct association between EA-230-induced gains in POC-induced morbidity and gains in LOS.

Table 3 A	Averages		
Below median per-operative treatment duration, range 95 minutes to 152 minutes.	POC Duration (hrs)	LOS-ICU (hrs)	LOS-Hos (days)
Placebo	5,6	33,3	12,6
EA-230	1,2	20,4	9,5
Gain*	4,4	12,9	3,1
Table 3 B	Averages		

Above median per-operative treatment duration, range 156 minutes to 249 minutes	POC Duration (hrs)	LOS-ICU (hrs)	LOS-Hos (days)
Placebo	8,6	98,7	13,6
EA-230	1,6	37,5	8,4
Gain*	7	61,2	5,2

Figure 5 builds further on the as calculated with 71 patients experiencing mild, moderate, and severe SOOMs of defined duration. For placebo patients, the data shown corroborate the established observations in the art that the longer the operation lasts, the longer the patient stays in the hospital. In particular, the prolonged operative time that is found [13] is now herein associated with an increase in the risk of vascular complications and tissue damage that lead to the here increased duration of POC and increased length-of-stay (LOS) in the hospital. As indeed can be observed from the trendline plotted along the with scatter plots, actually prolonging operation time prolongs LOS. Following that rising trendline, table 4 has calculated an ~ 0.5 -day increase in LOS per every half hour extension of operating time. However, treatment with an AIP according to the invention reverses the rising of the trendline into a lowering, therewith reflecting an actual shortening of the length of the stay in the hospital due to treatment with AIP. As can be seen in Table 4, an ~ 0.3 -day decrease in LOS per every half hour extension of operating time is calculated. These findings translate into a gain of at around 0.8 day decrease in LOS per every half hour treatment with AIP versus placebo, in particular after the patient has received treatment already 1 hour, up to at around 4 to 5 hours of treatment.

Table 4 Calculation of gain (days) in LOS-Hos per 30 minutes per-operative treatment with EA-230 versus placebo.			
IMP (minutes)	LOS-Hos (days) placebo	LOS-Hos (days) EA-230	Gain
30	10,63	10,34	0,3
60	11,18	10,02	1,2
90	11,74	9,71	2,0

120	12,30	9,39	2,9
150	12,86	9,07	3,8
180	13,42	8,75	4,7
210	13,97	8,43	5,5
240	14,53	8,11	6,4
270	15,09	7,79	7,3
300	15,65	7,47	8,2

Analyses of potential cost-reduction after use of AIP AQGV, as based on Phase IIb results and calculated according to 2024 US pricing.

- 5 Cost estimates relating to postoperative complications (POC) in a US healthcare setting were found in PubMed and other published sources and adjusted for healthcare-specific inflation to 2024 US pricings (Health Care Inflation in the United States (1948-2024)).
<https://www.usinflationcalculator.com/inflation/health-care-inflation-in-the-united-states>.
 (Accessed March 1, 2024).

10 .

- Using data from the Michigan Surgical Quality Collaborative, Healy et al (2016[6]) analyzed 5120 patient episodes of surgical care. Overall, 744 patients (14.5%) experienced POC. They found unadjusted mean hospital costs (excluding professional physician fees) to be \$41.942,- higher ($P < .001$) for patients with POC (\$59.205,-) compared with those without POC
 15 (\$17.263,-). Adjusted to 2024 US pricing, this cost is \$53.490,- higher for patients with POC.

- These findings compare well with those of Eappen et al (2013[89]) who studied 34256 surgical discharges during 2010 from a nonprofit 12-hospital system in the southern United States. Again, physician expenses were not collected. POC were associated with a \$39.017 ($P < .001$) higher hospital cost per patient. At inflation-adjusted 2024 US pricing, this cost is
 20 \$55.839,- higher for patients with POC.

Whereas P-values of the Phase IIb study are established on non-parametric statistics and medians, herein (differences in) costs are estimated on means and differences between means. It is estimated that EA-230 may contribute to a cost-reduction of \$33.420,- per major surgery patient in a US setting.

5 COST-REDUCTION ANALYSES.

To estimate the potential of EA-230 related US health-care cost-reductions apparent from the Phase IIb study, three individual cost-factors of significant therapeutic effects of EA-230 were identified, adjusted for healthcare-specific inflation to reflect 2024 US pricing, and totaled. Length-of-stay (LoS) in ICU and hospital are generally considered fixed costs whereas
10 physician expenses, drug treatment and nursing labor costs are generally considered variable costs.

FIXED COST ESTIMATES.

ICU				Hospital			
Estimated costs*				Estimated costs**			
\$8.686,-/day*				\$3.046,-/day			
Phase IIb study				Phase IIb study			
Placebo		EA-230		Placebo		EA-230	
40		25		248		203	
hours		hours		hours		hours	
		0.625 day				1,875 days	
Cost reduction				Cost reduction			
\$5.428,75				\$5.711,25			
				Total fixed-cost reduction EA-230			
				\$11.140,-			

*Based on Dasta et al. 2005, inflation adjusted

**Based on American Hospital Association Survey 2021, inflation adjusted

LoS in ICU ($p = 0.02$): In the Phase IIb study, EA-230 treated CABG-patients required on average 25 hrs. LoS in the ICU whereas placebo treated patients required on average 40 hrs. LoS in ICU thus demonstrating a 15-hour difference ($40 - 25$), which corresponds to a 0.625-day difference in favor of EA-230.

- 5 Starting point for the calculation were daily costs of stay at an US-ICU setting (without mechanical ventilation) as determined by Dasta et al (2005[16]); a study including 51.009 adult patients admitted between October 1, 2002, and December 31, 2002, to an ICU of one of 253 geographically distinct US hospitals. ICU costs are reported highest during the first 2 days of admission (\$5.082/day), stabilizing at a lower level thereafter (\$3.184/day).
- 10 Since on average patients in the Phase IIb study left at around day 2, the figure of \$5.082/day was taken and inflation-adjusted from 2005 to 2024 US pricing (\$8.686-/day). To allow for a conservative estimate, inflation was adjusted from the year of publication. The difference between placebo and EA-230 (0.625 day) was then applied, resulting in an estimated \$5.429,- reduction in costs for LoS in ICU for EA-230 treated patients.
- 15 LoS in hospital ($p = 0.001$): EA-230 patients required on average 9.5 days (228 hrs.) stay in the hospital, where placebo patients required on average 12 days (288 hrs.). LoS in hospital thus demonstrating a $((288-40 \text{ ICU hrs.}) - (228 - 25) = 45 \text{ hrs.}) = 1.875\text{-days}$ difference in favor of EA-230. Starting point for the calculation were daily costs of stay per inpatient day in a US hospital in 2021, as determined (<https://www.beckershospitalreview.com/finance/hospital-expenses-per-inpatient-day-across-50-states-2023.html>) from the 2021 American Hospital Association Annual Survey for every US state, with a national average for nonprofit hospitals at \$3.013,-/day.
- 20

- This figure was inflation adjusted from 2023 to 2024 US pricing (\$3.046,-), and then applied to the difference between placebo and EA-230 (1.875 day), resulting in an estimated
- 25 \$5.711,- reduction in costs for LoS in hospital for EA-230.

Thus, fixed cost-estimates of LoS in ICU and hospital taken together amount to $(\$5.429 + \$5.711 =)$ a \$11.140,- reduction for EA-230, at 2024 US pricing.

VARIABLE COST ESTIMATES

Costs of treatment-dependent POC ($p = 0.0026$): EA-230-treated patients showed a median treatment-dependent POC-duration of 17.2 hrs versus placebo-treated patients 44.3 hrs in the Phase IIb study. However, additional drug costs, nursing labor costs nor physician expenses were tallied in the study, thus no straightforward approach to directly calculate variable costs is available from the Phase IIb data. Herewith an indirect estimate of differences in variable costs is provided.

Canadian (Roach et al, 2022[9]) as well as US (Kachare et al, 2015[90]) studies provide a ratio between variable versus fixed hospital costs of 2-to-1 and 5-to-2 respectively, not counting physician expenses. For a conservative approach the 2:1 ratio was applied to the above identified gain in fixed costs/patient. The difference of variable costs of POC between placebo and EA-230 treated patients is appreciated at ($2 \times \$11.140,- =$) \$22.280,-, at 2024 US pricing in favor of EA-230.

SUM OF FIXED AND VARIABLE COST ESTIMATES

From the sum of fixed costs + variable costs an estimated cost-reduction after treatment with EA-230 arises of \$33.420,-. Actual findings of US-costs of POC corroborate the order of magnitude of the above cost-reduction findings. As detailed above, at 2024 US pricing two US studies (Healy et al and Eapen et al) independently show at around \$55.000,- higher costs associated with patients experiencing POC.

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Claims

- 1 A source of autophagy-inhibiting amino acids for parenteral application as a medicament for use at least per-operatively for the treatment of the vascular system to reduce duration of a post-operative complication (POC) in a subject,
5 wherein each of said amino acids is selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R).
- 2 The source for use according to claim 1 provided as an autophagy-inhibiting-peptide (AIP).
- 10 3 The source for use according to claim 2 wherein said peptide comprises at least 3, preferably at least 4 amino acids.
- 4 The source for use according to any of claims 1 to 3 for use to reduce duration of moderate or severe post-operative complications in a subject.
- 5 The source for use according to any of claims 1 to 4 wherein said POC comprise
15 infection.
- 6 The source for use according to any of claims 1 to 5 wherein said per-operative treatment lasts at least 30 minutes.
- 7 The source for use according to any of claims 1 to 6 wherein said per-operative treatment lasts at least 95 minutes.
- 20 8 The source for use according to any of claims 1 to 7 wherein said per-operative treatment is preceded by a pre-operative (before surgery) parenteral treatment with said source.
- 9 The source for use according to any of claims 1 to 8 wherein said per-operative treatment is followed by a post-operative (after surgery) parenteral treatment
25 with said source.
- 10 The source for use according to any of claims 1 to 9 wherein each of said autophagy-inhibiting amino acids are selected from the group consisting of A, Q, G, V, L and P.
- 11 The source for use according to any of claims 1 to 10 wherein each of said
30 autophagy-inhibiting amino acids is selected from the group consisting of A, Q, L and P.

- 12 The source for use according to any of claims 1 to 11 that at least comprises a dipeptide selected from the group AQ, QQ, LQ, GQ, PQ, VQ, AL, LL, QL, GL, PL, PA, VL, AL, PQ, QA, QL, QG, QP, QV, LA, LG, LP, and LV.
- 5 13 The source for use according to any of claims 1 to 12 that at least comprises a tripeptide selected from the group AQG, QQG, LQG, GQG, PQG, VQG, ALG, LLG, QLG, GLG, PLG, VLG, VLA, VLP, PAL, ALP, QAG, QLG, QGG, QPG, QVG, LAG, LGG, LPG, and LVG.
- 10 14 The source for use according to any of claims 1 to 13 at least comprises a tetrapeptide selected from the group AQGV, QQGV, LQGV, GQGV, PQGV, VQGV, ALGV, LLGV, QLGV, GLGV, PLGV, VLGV, QAGV, QLGV, QGGV, QPGV, QVG, LAGV, LGGV, LPGV, VLPA, LPAL, PALP and LVGV.
- 15 15 A solution for intravenous administration comprising a source of amino acids according to any of claims 1 to 14.
- 16 16 A method of medical treatment for reducing duration, preferably cumulative duration of post-operative complications (POC) in a subject, said treatment comprising providing said subject at least during surgery with a source of autophagy-inhibiting amino acids, wherein each of said amino acids is selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R).
- 20 17 The method according to claim 16 for reducing duration of moderate and severe POC.
- 18 18 The method according to claims 16 or 17 wherein said source of autophagy-inhibiting amino acids is an autophagy-inhibiting peptide (AIP) comprising said autophagy-inhibiting amino acids.
- 25 19 The method according to anyone of claims 16 to 18 wherein the method further comprises providing said source of autophagy-inhibiting amino acids to said subject prior to surgery, preferably wherein said subject is deemed suffering from pre-operative sepsis or pre-operative injuries and requires surgery.
- 30 20 The method according to anyone of claims 16 to 19 wherein the method further comprises providing said source of autophagy-inhibiting amino acids to said subject after surgery, preferably wherein said subject is deemed suffering from a systemic inflammatory response after surgery.

- 21 The method according to any of claims 16 to 20 wherein each of said autophagy-inhibiting amino acids is selected from the group consisting of A, Q, G, V, L and P.
- 22 The method according to any of claims 16 to 21 wherein each of said autophagy-inhibiting amino acids is selected from the group consisting of A, Q, L and P.
- 5 23 The method according to any of claims 16 to 22 wherein said source of autophagy-inhibiting amino acids at least comprises a dipeptide selected from the group AQ, QQ, LQ, GQ, PQ, VQ, AL, LL, QL, GL, PL, PA, VL, AL, PQ, QA, QL, QG, QP, QV, LA, LG, LP, and LV.
- 24 The method according to any of claims 16 to 23 wherein said source of
10 autophagy-inhibiting amino acids at least comprises a tripeptide selected from the group AQG, QQG, LQG, GQG, PQG, VQG, ALG, LLG, QLG, GLG, PLG, VLG, VLA, VLP, PAL, ALP, QAG, QLG, QGG, QPG, QVG, LAG, LGG, LPG, and LVG.
- 25 The method according to any of claims 16 to 24 wherein said source of autophagy-inhibiting amino acids at least comprises a tetrapeptide selected from
15 the group AQGV, QQGV, LQGV, GQGV, PQGV, VQGV, ALGV, LLGV, QLGV, GLGV, PLGV, VLGV, QAGV, QLGV, QGGV, QPGV, QVG, LAGV, LGGV, LPGV, VLPA, LPAL, PALP and LVGV.
- 26 A source of autophagy-inhibiting amino acids as a medicament for use in a method of treatment according to any one of claims 16 to 25, wherein each of
20 said amino acids is selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R).
- 27 An autophagy-inhibiting-peptide (AIP) comprising autophagy-inhibiting amino acids as a medicament for use in a method of treatment according to any one of
25 claims 16 to 25, wherein each of said amino acids is selected from the group consisting of alanine (in one letter code: A), glutamine (Q), glycine (G), valine (V), leucine (L), isoleucine (I), proline (P) and arginine (R).
- 28 A solution for intravenous administration comprising a source of amino acids according to claim 15 for use in a method of treatment according to any one of
30 claims 16 to 27.