S7-21). The recruitment of progenitor cells is divided in four phases: mobilisation, migration, invasion and engraftment of the cells to the fracture site. Impairment of *inter alia* any one or more of the above processes can result in impaired bone fracture healing.

The diagnosis is currently radiologic and is made after 3 to 4 months without consolidation for delayed unions and after 6 to 9 months without consolidation for non-unions, depending on the site and type of fracture (Frolke et al. *Supra*). Clinically useful, biomarker-based screening tests for impaired bone fracture healing are, to our knowledge, not available. In fact, the use of biological molecules as biomarkers for impaired bone fracture healing has never been investigated in non-union patients (NU). In pathophysiological studies, authors have demonstrated that *in situ* TGF-β, PDGF, FGF, and BMP 2/4 levels were the same in NU and healthy subjects 1 week after trauma, but were decreased 8 weeks later in the NU patients (Brownlow et al. *Injury*, 2001, vol. 32(7), 519-524). Other authors have shown that serum levels of TGF-β, PDGF-AB and FGF were lower at 2 and 4 weeks after trauma in NU patients. TGF-β1 and PDGF-AB were still lower in NU patients at 12 weeks after trauma (Weiss et al. *Arch. Orthop. Trauma Surg.*, 2009, vol. 129, 989-997; Zimmermann et al. *Bone*, 2005, vol. 36(5), 779-785).

Because knowledge, indication or warning that a fracture in a subject is or has an increased chance to show impaired healing, such as to progress towards a non-union, may aid therapeutic interventions in the subject, the provision of further, alternative and preferably improved methods and means for diagnosis, prediction, prognosis and/or monitoring of impaired bone fracture healing continues to be of prime importance.

SUMMARY ~~OF THE INVENTION~~

Having conducted extensive experiments and tests, the inventors identified biological molecules whose levels are closely predictive and/or indicative of impaired healing of a bone fracture, and which thus constitute useful and promising biomarkers for impaired fracture healing. The synonymous phrases "impaired bone fracture healing" or "impaired fracture healing" as used herein encompass any anomalies, abnormalities and deficiencies of bone fracture healing, such as inadequate, delayed or absent bone fracture healing. The phrases intend to specifically comprise and preferably denote mal-unions, delayed unions and non-unions, more preferably denote non-unions, including *inter alia* tight non-unions and unstable non-unions (pseudarthrosis).

In accordance with the disclosure~~invention~~, additional and markedly improved methods and means for diagnosis, prediction, prognosis and/or monitoring of impaired fracture healing are realised through the provision of any one or more of stromal derived factor-1 (SDF-1 or CXCL12), platelet-derived growth factor BB

(PDGF-BB), interleukin-8 (IL-8 or CXCL8) and interleukin-6 (IL-6) as biomarker(s) for impaired bone fracture healing.

Hence, provided herein is use of any one or more of SDF-1, PDGF-BB, IL-8 and IL-6 as a biomarker, more particularly as a biomarker for impaired fracture healing, even more particularly as a biomarker for the diagnosis, prediction, prognosis and/or monitoring of impaired fracture healing. Preferably, provided herein is use of IL-8 as a biomarker, more particularly as a biomarker for impaired bone fracture healing, even more particularly as a biomarker for the diagnosis, prediction, prognosis and/or monitoring of impaired bone fracture healing.

In one aspect, there is provided an *in vitro* use of interleukin-8 (IL-8) as a biomarker for impaired bone fracture healing.

Also provided is use of any one or more of SDF-1, PDGF-BB, IL-8 and IL-6 for the diagnosis, prediction, prognosis and/or monitoring of impaired fracture healing. In one aspect, there is provided the~~Preferably, provided herein is~~ use of IL-8 for the in vitro diagnosis, prediction, prognosis and/or monitoring of impaired bone fracture healing.

In preferred embodiments of the present uses, the impaired bone fracture healing may be selected from the group consisting of mal-union fracture, delayed union fracture, and non-union fracture.

Provided herein is as well a method for the diagnosis, prediction, prognosis and/or monitoring of impaired fracture healing in a subject comprising measuring the level of any one or more of SDF-1, PDGF-BB, IL-8 and IL-6 in a sample from said subject. In one aspect, there is provided~~Preferably, provided herein is~~ a method for the diagnosis, prediction, prognosis and/or monitoring of impaired bone fracture healing in a subject comprising measuring the level of IL-8 in a sample from said subject. To measure the level of one or more biomarkers, the present methods, and particularly the examination phase of such methods in which data is collected from and/or about the subject, typically comprise measuring or determining the level (i.e., quantity, amount) of said one or more biomarker(s) in a sample from the subject.

A method for the diagnosis, prediction and/or prognosis of impaired fracture healing in a subject as taught herein may in preferred embodiments comprise steps: (i) measuring the quantity of any one or more of SDF-1, PDGF-BB, IL-8 and IL-6 in a sample from the subject; (ii) comparing the quantity as measured in (i) with a reference value representing a known diagnosis, prediction and/or prognosis of impaired fracture healing; (iii) finding a deviation or no deviation of the quantity as measured in (i) from the reference value; and (iv) attributing said finding of deviation or no deviation to a particular diagnosis, prediction and/or prognosis of impaired fracture healing in the subject. Preferably, a method as taught herein for the diagnosis, prediction and/or prognosis of impaired bone fracture healing in a subject may comprise steps: (i) measuring the quantity of IL-8 in a sample from the subject; (ii) comparing the quantity as measured in (i) with a reference value representing a known diagnosis, prediction and/or prognosis of impaired bone fracture healing; (iii) finding a deviation or no deviation

successive time points compared to the respective quantity at an earlier one of said two or more successive time points, can indicate that the subject's risk of developing impaired fracture healing has increased.

Also disclosed is a method to determine whether a subject is or is not (such as, e.g., still is, or is no longer) in need of a therapeutic or prophylactic (preventative) treatment of impaired fracture healing comprising measuring the level of any one or more of SDF-1, PDGF-BB, IL-8 and IL-6 in a sample from said subject. In preferred embodiments the method may comprise steps: (i) measuring the quantity of any one or more of SDF-1, PDGF-BB, IL-8 and IL-6 in the sample from the subject; (ii) comparing the quantity as measured in (i) with a reference value representing a known diagnosis, prediction and/or prognosis of impaired fracture healing; (iii) finding a deviation or no deviation of the quantity as measured in (i) from the reference value; (iv) inferring from said finding the presence or absence of a need for a therapeutic or prophylactic treatment of impaired fracture healing. In one aspect, there is provided~~Preferably~~, a method to determine whether a subject is or is not in need of a therapeutic or prophylactic treatment of impaired bone fracture healing ~~may~~ comprise~~ing~~ measuring the level of IL-8 in a sample from said subject, preferably ~~may~~ comprise~~ing~~ steps: (i) measuring the quantity of IL-8 in the sample from the subject; (ii) comparing the quantity as measured in (i) with a reference value representing a known diagnosis, prediction and/or prognosis of impaired bone fracture healing; (iii) finding a deviation or no deviation of the quantity as measured in (i) from the reference value; (iv) inferring from said finding the presence or absence of a need for a therapeutic or prophylactic treatment of impaired bone fracture healing. A treatment may be particularly indicated where the method allows for a conclusion that the subject has impaired fracture healing or is at risk of having impaired fracture healing or has a poor prognosis for impaired fracture healing.

In this context further disclosed is a method for determining the outcome of a therapeutic or prophylactic treatment of impaired fracture healing in a subject, comprising measuring the level of any one or more of SDF-1, PDGF-BB, IL-8 and IL-6, preferably the level of IL-8, in a sample from said subject.

In preferred embodiments of the present methods, the impaired bone fracture healing may be selected from the group consisting of mal-union fracture, delayed union fracture, and non-union fracture.

Treatments of impaired fracture healing include, *inter alia*, non-invasive treatments such as, e.g., electrical stimulation, ultrasounds or specialized braces, and invasive measures, such as, e.g., surgical removal of dead tissue, insertion of internal brace (e.g., rod, plate or screw), insertion of bone graft, injection of one or more bone morphogenetic proteins (BMPs), or amputation to prevent further injury.

(b) a prediction or diagnosis of impaired fracture healing or a poor prognosis for impaired fracture healing,

comprising:

(i) measuring the quantity of the one or more biomarkers in a sample from:

(i a) one or more subjects not having impaired fracture healing or not being at risk of having impaired fracture healing or having a good prognosis for impaired fracture healing, or

(i b) one or more subjects having impaired fracture healing or being at risk of having impaired fracture healing or having a poor prognosis for impaired fracture healing, and

(ii a) establishing from the quantity of the one or more biomarkers as measured in (i a) the reference value representing the prediction or diagnosis of the absence of impaired fracture healing or representing the good prognosis for impaired fracture healing, or

(ii b) establishing from the quantity of the one or more biomarkers as measured in (i b) the reference value representing the prediction or diagnosis of impaired fracture healing or representing the poor prognosis for impaired fracture healing.

In one aspect, there is provided~~Preferably, also provided herein is~~ a method for establishing a reference value for IL-8, said reference value representing:

(a) a prediction or diagnosis of the absence of impaired bone fracture healing or a good prognosis for impaired bone fracture healing, or

(b) a prediction or diagnosis of impaired bone fracture healing or a poor prognosis for impaired bone fracture healing,

comprising:

(i) measuring the quantity of IL-8 in a sample from:

(i a) one or more subjects not having impaired bone fracture healing or not being at risk of having impaired bone fracture healing or having a good prognosis for impaired bone fracture healing, or

(i b) one or more subjects having impaired bone fracture healing or being at risk of having impaired bone fracture healing or having a poor prognosis for impaired bone fracture healing, and

values represent a known diagnosis, prediction and/or prognosis of impaired bone fracture healing. Further provided is the use as taught herein, wherein means for collecting a sample from a subject is provided separately from or is comprised in the kit.

In one aspect, there is provided the use of a kit for the diagnosis, prediction, prognosis and/or monitoring of impaired bone fracture healing in a subject, the kit comprising means for measuring the quantity of IL-8, particularly in a sample from the subject.

Also disclosed are reagents and tools useful for measuring any one or more biomarkers as taught herein. Hence, disclosed is a nucleic acid array or microarray or a protein, polypeptide or peptide array or microarray comprising any one, preferably any two, preferably any three, more preferably all four of SDF-1, PDGF-BB , IL-8 and/or IL-6 . Also disclosed is a binding agent array or microarray comprising one or more binding agents capable of specifically binding to any one, preferably any two, preferably three, more preferably all four of SDF-1, PDGF-BB , IL-8 and/or IL-6 , preferably comprising a known quantity or concentration of said one or more binding agents.

Further disclosed is the use of any one array or microarray as described herein for the diagnosis, prediction, prognosis and/or monitoring of impaired fracture healing.

In one aspect, there is provided the~~Preferably, provided is~~ use of a nucleic acid array or microarray or a protein, polypeptide or peptide array or microarray for the diagnosis, prediction, prognosis and/or monitoring of impaired bone fracture healing, said array or microarray comprising a nucleic acid encoding IL-8 or comprising IL-8. Preferably, also provided is use of a binding agent array or microarray for the diagnosis, prediction, prognosis and/or monitoring of impaired bone fracture healing, said array or microarray comprising one or more binding agents capable of specifically binding to IL-8, preferably comprising a known quantity or concentration of said one or more binding agents.

In one aspect, there is provided the use of a binding agent array or microarray for the diagnosis, prediction, prognosis and/or monitoring of impaired bone fracture healing, said array or microarray comprising one or more binding agents capable of specifically binding to IL-8.

The above and further embodiment~~aspects~~ and preferred embodiments of the disclosure~~invention~~ are described in the following sections and in the appended claims. The subject matter of appended claims is hereby specifically incorporated in this specification.

BRIEF DESCRIPTION OF FIGURES

Figure 1 illustrates plasma levels of SDF-1 in an experiment comparing a group of non-union patients (NU) with healthy controls (HV), (A) all samples (HV, n = 49; NU, n = 15), (B) samples where plasma is collected in heparin tubes (HV, n = 26; NU, n = 11), (C) samples where plasma is collected in EDTA tubes (HV, n = 40; NU, n = 5).

Figure 2 illustrates serum levels of PDGF-BB in an experiment comparing a group of non-union patients (NU, n = 9) with healthy controls (HV, n = 20).

Figure 3 illustrates serum levels of IL-8 in an experiment comparing a group of non-union patients (NU, n = 4) with healthy controls (HV, n = 18).