A 32-month follow-up study of nanovesicle concentrations in blood from 12 patients with gastrointestinal stromal tumour treated with imatinib

Eva Ogorevc*, Roman Štukelj†, Apolonija Bedina-Zavec‡, Vid Šuštar§, Metka Šimundić†, Veronika Kralj-Iglič†[1] and Rado Janša‖

*Laboratory of Biophysics, Faculty of Electrical Engineering, University of Ljubljana, Tržaška 25, SI-1000 Ljubljana, Slovenia, †Biomedical Research Group, Faculty of Health Sciences, University of Ljubljana, Zdravstvena 5, SI-1000 Ljubljana, Slovenia, ‡Laboratory of Biosynthesis and Biotransformation, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia, §Laboratory of Clinical Biophysics, Chair of Orthopaedic Surgery, Faculty of Medicine, University of Ljubljana, Lipičeva 2, SI-1000, Ljubljana, Slovenia, and ‖Clinical Department of Gastroenterology, Ljubljana University Medical Centre, Zaloška cesta 7, SI-1000 Ljubljana, Slovenia

Abstract

Clinical studies have indicated that the NV (nanovesicle) concentration in blood samples is a potential indicator of clinical status and can be used to follow the development of the disease. For 32 months, we monitored the effect of imatinib treatment on NV concentrations in blood samples from 12 patients with GIST (gastrointestinal stromal tumour). The NV concentration before the treatment increased with respect to control by a factor of 3.5 on average (range 2.6–9.2). The first week after initiation of the treatment, the NV concentration increased considerably, by a factor of 13 on average (range 5.9–21.2), whereas on average, after 1 month, it decreased to the level of the control and remained at that level for at least 1.5 years. Recent assessment (after 2.5 years) showed a somewhat increased NV concentration, by a factor of 2 on average (range 0.7–3.9). Low NV concentrations in blood samples during the treatment reflect a favourable effect of imatinib in these patients and no remission of the disease was hitherto observed.

Cell NVs (nanovesicles) isolated from blood: a clinically relevant artefact

Cell NVs are sub-micron-sized membrane-enclosed particles that are shed from cells of all types into the surrounding solution in the last stage of the budding process [1,2]. It is expected that NVs can be isolated from body fluids and used for diagnostic purposes [3–7]. In order to be assessed, NVs must be harvested from blood samples, usually by centrifugation and washing of samples. Their concentration can be determined by flow cytometry [8–12]. It was shown that, in some diseases including cancer, the NV concentration in blood samples was increased with respect to the samples of healthy subjects [13–17]. On the basis of imaging of isolates from blood [2,18] and experiments where external parameters were varied during the isolation [18], it was suggested that the particles obtained by centrifugation and washing are largely blood cell (platelet) fragments which are created after blood sampling. Therefore they are an artefact, yet they are clinically relevant, since they reflect vesiculability of blood cells (mostly platelets) and physical properties of plasma which seem to be changed in disease [15]. It would be of ultimate interest to monitor the composition and potential biological impact of NVs in isolates from blood. However, choices of relevant biomarkers are under development and validation, whereas understanding of processes leading to the population of NVs in isolates is still rudimentary. Therefore the NV concentration in blood samples currently provides useful information on the effect of the treatment and/or development of the disease.

Treatment of GISTs (gastrointestinal stromal tumours) by imatinib

GIST is the most common sarcoma of the gastrointestinal tract. It is mostly characterized by mutations in KIT or PDGFR (platelet-derived growth factor receptor) tyrosine kinase receptors involved in tumour proliferation [19,20]. Imatinib (also known as STI571, Glivec or Gleevec; Novartis Pharmaceuticals) binds competitively to the binding site for ATP of some tyrosine kinases and inhibits their signalling [21]. Thereby it suppresses survival and proliferation of tumour cells [22,23]. Imatinib improved the prognosis of locally advanced inoperable or metastatic GISTs [24] as well as of stable disease [25] and is currently considered standard therapy for GISTs, in particular those attributed to known genetic defects [20]. It was found that the treatment induces changes in the tumour structure (e.g. decreases tumour

Key words: exosome, gastrointestinal stromal tumour (GIST), imatinib, microparticle, microvesicle.

Abbreviations used: GIST, gastrointestinal stromal tumour; MDCK, Madin–Darby canine kidney; NV, nanovesicle.

[1]To whom correspondence should be addressed (email veronika.kralj-iglic@fe.uni-lj.si).

vascularity, haemorrhage and necrosis) [26]. As these features could be connected to cell vesiculation, it is suggested that treatment with imatinib may affect vesiculation which would be reflected in the NV concentration in blood isolates.

Hypothesis

It was the aim of our study to investigate the effect of treatment of GIST with imatinib on the NV concentration in blood samples. The study included 12 patients who were treated by imatinib at the Department of Gastroenterology, Ljubljana University Medical Centre, Slovenia, from 2009. The patient data are shown in Table 1. The study included six patients with previously resected GIST who started treatment with imatinib the same day. In this group, NVs were assessed before the initiation of treatment (zero time) and followed for 128 weeks (2.5 years). The study also included six patients with previously resected tumour who started taking imatinib before the beginning of the study, but joined the study approximately 2 months after the study begun. To calibrate the results, blood was donated by one of the authors in all experiments (female, 51 years, with no record of disease). All subjects gave informed consent to the study which was approved by the Slovenian National Medical Ethics Committee (number 117/02/10). The study conformed to the ethical principles given in the Declaration of Helsinki.

For isolation of NVs, 2.7 ml of blood was taken by vein puncture from a medial cubital vein into evacuated tubes (BD Vacutainers, Becton Dickinson) containing 0.109 M buffered trisodium citrate. A 21-gauge needle (length 70 mm, inner radius 0.4 mm) (Microlance, Becton Dickinson) was used. Samples were processed within 15 min. Up to eight samples were processed together, to minimize artefacts caused by keeping the samples waiting.

After sampling, blood was kept at 37°C until insertion into the centrifuge. Blood was centrifuged in a Centric 400R centrifuge (Domel) using an RS 4/100 swing-out rotor at 1550 ***g*** sustained acceleration for 15 min at 37°C. Then, 250 μl of plasma was removed from the top of the Vacutainer and inserted into a 1.5 ml Eppendorf tube. Samples were centrifuged at 17570 ***g*** sustained acceleration for 30 min at 37°C in a Centric 200R centrifuge using an RA24 angle rotor. The choice of temperature is a modification with respect to the original protocol [27] where centrifugation and isolation would have been performed at room temperature. Then, 225 μl of supernatant was removed. The pellet was resuspended in 225 μl of PBS (137 mM NaCl, 2.7 mM KCl, 7.8 mM $Na_2HPO_4 \cdot 2H_2O$ and 1.5 mM KH_2PO_4, pH 7.4) containing 10.9 mM trisodium citrate (PBS-citrate). Samples were vortex-mixed for 10 s and centrifuged at 17570 ***g*** sustained acceleration for 30 min at 37°C in the Centric 200R centrifuge using an RA24 angle rotor. To prepare samples for flow cytometry, 225 μl of supernatant was removed, 75 μl of PBS-citrate was added to the pellet and vortex-mixed for 10 s.

Taking blood with Vacutubes yields variations in volume of blood (up to ±20%), which is important, especially as this causes differences in the distance of the level of blood from the centrifuge rotor axis. We assumed linear dependence of the number of NVs isolated and volume of the sample, and corrected the number of NVs to the supposed 2.7 ml in all samples by dividing the measured number of NVs by the actual volume of blood and multiplying the result by 2.7 ml.

Counting of NVs in isolates and estimation of their average size was performed using an Altra Flow Cytometer instrument (Beckman Coulter) by FS (forward scatter) and SS (side scatter) parameters. For measurement of the NV concentration, 20 μl of calibrating microspheres (Flow Count, Beckmann Coulter) of 10 μm size and known concentration (1.05×10^6/ml) were diluted in an appropriate volume of PBS-citrate and added to the samples. At least 10000 events were recorded for each sample. The results are given by the dimensionless ratio between the number of events corresponding to NVs and the number of events corresponding to NVs of the control (subject with no record of disease).

Effect of treatment with imatinib due to GIST on NVs isolated from blood

Table 1 shows the time-dependence of the NV concentration in blood isolates of patients treated with imatinib (relative to a control with no record of disease). Before treatment, the NV concentration was elevated with respect to control by a factor of 3.5 (range 1–9.2). The concentration was higher than in control in four out of six patients. The first week after the treatment, the NV concentration in all patients increased considerably with respect to before the treatment. Concomitantly, five out of six patients reported side effects. The increase of the concentration with respect to the control was very high, a factor of 13.5 on average (range 5.9–21.2). However, after 1 month, the NV concentration in patients decreased to the level of the control and remained at that level or below for 1.5 years. In all measurements within this time, the majority of patients had NV concentrations lower than that of the control. The last assessment showed somewhat elevated NV concentrations in patients, by a factor of 2 (range 0.7–3.9). Also, six out of nine patients had higher NV concentrations than that of the control. The results presented were calibrated to the same subject in all measurements, which seemed the best available solution at this point of the development of the method. The concentration may vary in the control subject and small or moderate differences in average values could originate from an inaccuracy of the method. However, variations within the same subject are unlikely to cause the ratio between the NVs of the patient and of the control to be 5 or more (which was the case in the first week after initiation of treatment).

To study the effect of the centrifugation on the NV concentration in isolates from blood, we gathered two pools of plasma after the first centrifugation in which blood cells were divided from plasma: the uppermost 250 μl and the next 250 μl. Figure 1 shows the median size of NVs in both pools (upper and lower) for six patients (2, 4, 5, 6, 8 and 9; see Table 1) with GIST and for the control. The

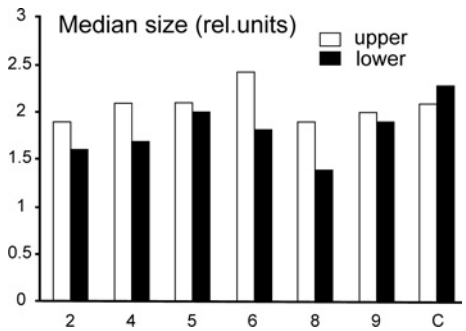
Table 1 | **NV concentrations in blood samples (relative to NV concentrations in a subject with no record of disease) of 12 patients with GIST treated with imatinib**

Patients 1–6 were assessed before starting the treatment (week 0) and during the treatment (weeks 1–128); patients 7–12 started receiving imatinib before the beginning of the study. Side effects: oe, oedema; mp, muscle pain; r, rash; n, nausea. The final row presents average values for patients that were present at sampling at a given time. Not all patients that were invited to sampling were able to respond at all dates. The same control subject donated blood for all experiments.

Patient data									Week of treatment							
Patient	Type of tumour	Start of therapy (year)	Number of divisions	Dose of drug (mg)	Size of tumour (cm)	Location of tumour	Metastases	Side effect	0	1	5	13	23	32	80	128
1	GIST, exon 11	2009	4	400	5	Stomach	–	oe, mp, r	2.6	21.2	1.28	–	–	–	–	–
2	GIST, exon 11	2009	10	400	11	Stomach	–	oe, n	2.6	14.5	0.9	0.4	0.68	0.32	0.82	–
3	GIST, wild-type	2009	4	400	5.2	Stomach	–	oe, mp, r	9.2	10.8	1.82	–	0.23	1.2	0.64	3.25
4	GIST, wild-type	2009	10	400	6.5	Small intestine	–	–	3.2	–	2.7	0.64	2.06	1.23	1.39	0.98
5	GIST, exon 11	2009	10	400	4.5	Stomach	–	oe	1	5.9	0.6	0.42	–	0.24	0.52	0.99
6	GIST, exon 11	2009	10	400	3	Stomach	–	mp, n	2.3	14.8	1.4	0.66	–	0.7	1.75	3.89
7	GIST, exon 11	2009	10	400	2	Liver	+ removed	oe	–	–	–	–	0.5	–	–	1.52
8	GIST, exon 11	2009	4	400	1	Small intestine	–	–	–	–	–	0.78	–	–	–	1.19
9	GIST, exon 11	2009	4	400	4	Small intestine	–	–	–	–	–	0.91	1.17	–	–	0.67
10	GIST, exon 11	2007	10	400	7	Stomach	–	r, n	–	–	–	–	0.71	–	–	3.23
11	GIST, exon 11	2008	8	800	6.5	Stomach	–	oe, n	–	–	–	–	0.7	–	–	2.35
12	GIST, exons 11, 9	2008	2	400	4	Small intestine	–	–	–	–	–	–	0.5	–	–	–
Average									3.48	13.44	1.45	0.64	0.82	0.74	1.02	2.01

Figure 1 | Median size of NVs in isolates from blood of patients with GIST treated with imatinib

For each subject, NVs were isolated from the upper part of the supernatant after the first centrifugation (white bars) and from the lower part of the supernatant (black bars). Respective numbers assigned to patients in Table 1 are given below the bars. Patients and control (C) were assessed at week 13 after initiation of treatment with imatinib. The size was estimated by flow cytometry by the side-scattered light.

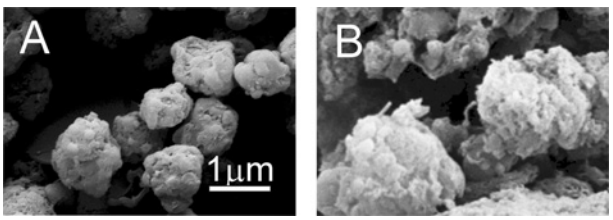


measurements were performed at week 13 after the beginning of the study. The median size of NVs in all patients was smaller in the lower plasma pool. In contrast, the median size of NVs in control was smaller in the uppermost plasma pool. If NVs were present in blood and isolated as they were, it could be expected that the lower plasma pool after the first centrifugation contained larger particles than did the upper pool. Namely, considering Stokes' law, the speed of sedimentation depends on the size of the particles and is higher for larger particles. In contrast with this, the median size of particles in the lower pool was found to be smaller in patients. This indicates that a large number of these particles was created during the centrifugation by fragmentation of platelets that probably resided in plasma after the first centrifugation. In patients, this effect was more pronounced than in the control, indicating changes in physical properties of the platelet membrane and/or plasma. The results presented in Figure 1 support the hypothesis that the majority of NVs found in blood isolates is created after blood sampling [18].

We tried to explain why the NV concentration increased dramatically after patients started taking imatinib. The peak in the concentration could be related to the presence of (residual) tumour cells in the body. Imatinib was shown to cause programmed cell death in GIST cells *in vivo* [28–32] and *in vitro* [28,29]. It was found recently that histone H2AX is involved in the induction of GIST cell death owing to imatinib treatment and can also lead to apoptosis in other cell types [33]. If apoptosis is induced, cells shed apoptotic bodies which could contribute directly to an increase in the NV concentration in isolates. Structures similar to apoptotic bodies originating from MDCK (Madin–Darby canine kidney) cells were found in blood of a patient with pancreatic cancer (Figure 2). However, it seems unlikely that the direct effect would be the only one contributing to a 5-fold or greater increase in the NV concentration in

Figure 2 | Apoptotic bodies obtained from cultured cells and from blood isolates

(**A**) Scanning electron micrograph of apoptotic bodies isolated from growth medium of MDCK cells. (**B**) Scanning electron micrograph of an isolate from blood of a patient with pancreatic cancer.



isolates. We speculate that material shed by dying cells can also interact with platelets and affect their elastic properties and therefore vesiculability. Our previous results showing that vesiculability of platelets and the properties of plasma determine the NV concentration in isolates [18], and our results (Figure 1) indicate that platelet and plasma properties probably contribute a major part to the NV concentration in isolates. Interestingly, the period of the dramatic increase in the NV concentration in the first week coincides with the lifespan of platelets. In addition, the possibility should be considered that, by affecting the tyrosine kinase receptors, imatinib induces changes in differentiation of haemopoietic stem cells to megakaryocytes, the progenitors of platelets [34].

Imatinib induces tumour cell death, but also tumour cell quiescence [35]. It has been suggested that imatinib can set the stage for future resistance by leading to a pool of cells that are not actively dividing, but may contain resistance mutations and could ultimately give rise to an imatinib-resistant clone [36]. The recent increase in NV concentration in patients by a factor of 2 (week 128) could indicate remission of the disease. Owing to poor accuracy of the method, such a conclusion cannot be certain on the basis of only one measurement, so further follow-up of patients is required.

Nevertheless, it seems unfavourable that a large amount of tumour-derived NVs would enter the circulation. The probability of interaction with other cells is higher if the concentration of tumour-derived NVs in circulation is higher. It follows from Table 1 that three patients (3, 5 and 6) were assessed both at week 1 when we observed a peak in NV concentration and at week 128. Higher NV concentrations at week 1 corresponded to higher concentration at week 128.

Non-specific biophysical mechanisms may be key to manipulation of nanovesiculation

Non-specific biophysical mechanisms are important in membrane budding and vesiculation. Formation of NVs is consistent with lateral distribution of membrane constituents and is reflected in the composition of NVs [37,38]. Therefore possible mechanisms of suppression of vesiculation should be considered. We have observed that plasma (or its

constituents) can mediate attractive interactions between membranes [39–41]. This was theoretically explained by attractive interaction mediated by plasma constituents [42]. A theoretical description of the system showed that a possible mechanism underlying the interaction is orientational ordering of molecules or complexes with internally distributed charge between the membranes in close proximity [43,44]. Owing to this mediated attractive interaction, membrane buds adhere to the mother cell and cannot become NVs which are free to move. It would therefore be of interest to study a potential role for imatinib in suppression of vesiculation also *in vitro* in model systems (giant phospholipid vesicles and blood cells).

Conclusions

Imatinib may cause a transient, but not long-term, increase in NV concentrations in blood isolates. Does this mean that increased vesiculability of platelets and/or increased NV concentration in blood could increase the risk of thromboembolic events in patients treated with imatinib at the beginning of the treatment? Tissue-factor-bearing platelet NVs were suggested to promote formation of blood clots in blood vessels [45,46]. However, aside from a case report of a lethal pulmonary embolism in a patient treated with imatinib which was explained as a consequence of occlusion of abdominal vein by necrotic tumour [47], to the best of our knowledge, there are no decisive reports of a connection of treatment with imatinib with thromboembolic disorders. Also, imatinib was shown to have a platelet anti-aggregation effect partly due to inhibition of fibrinogen binding to platelets [48]. On the basis of our results, anticoagulant prophylaxis in patients with GIST treated with imatinib is not indicated.

Acknowledgements

We thank the GIST patients for their collaboration and for donation of blood. We thank Peter Veranič from Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, for MDCK cells.

Funding

Financial support of the Slovenian Research Agency [grant numbers P2-0232, J3-4108 and J1-4109] and EUREKA initiative IMIPEB (Isolation of microvesicles from peripheral blood) is greatly appreciated.

References

1 Schara, K., Janša, V., Šuštar, V., Dolinar, D., Pavlič, J.I., Lokar, M., Kralj-Iglič, V., Veranič, P. and Iglič, A. (2009) Mechanisms for the formation of membranous nanostructures in cell-to-cell communication. Cell. Mol. Biol. Lett. **14**, 636–656

2 Mrvar-Brečko, A., Šuštar, V., Janša, V., Štukelj, R., Janša, R., Mujagić, E., Kruljc, P., Iglič, A., Hägerstrand, H. and Kralj-Iglič, V. (2010) Isolated microvesicles from peripheral blood and body fluids as observed by scanning electron microscope. Blood Cells Mol. Dis. **44**, 307–312

3 Kim, H.K., Song, K.S., Park, Y.S., Kang, Y.H., Lee, Y.J., Lee, K.R., Kim, H.K., Ryu, K.W., Bae, J.M. and Kim, S. (2003) Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor. Eur. J. Cancer **39**, 184–191

4 Skog, J., Würdinger, T., van Rijn, S., Meijer, D.H., Gainche1, L., Curry, Jr, W.T., Carter, B.S., Krichevsky, A.M. and Breakefield, X.O. (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat. Cell Biol. **10**, 1470–1476

5 Rabinowits, G., Gerçel-Taylor, C., Day, J.M., Taylor, D.D. and Kloecker, G.H. (2009) Exosomal microRNA: a diagnostic marker for lung cancer. Clin. Lung Cancer **10**, 42–46

6 Kosaka, N., Iguchi, H. and Ochiya, T (2010) Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci. **101**, 2087–2092

7 Baran, J., Baj-Krzyworzeka, M., Weglarczyk, K., Szatanek, R., Zembala, M., Barbasz, J., Czupryna, A., Szczepanik, A. and Zembala, M. (2010) Circulating tumour-derived microvesicles in plasma of gastric cancer patients. Cancer Immunol. Immun. **59**, 841–850

8 Zeiger, F., Stephan, S., Hoheisel, G., Pfeiffer, D., Ruehlmann, C. and Koksch, M. (2000) P-selectin expression, platelet aggregates, and platelet-derived microparticle formation are increased in peripheral arterial disease. Blood Coagulation Fibrinolysis **11**, 723–728

9 Robert, S., Poncelet, P., Lacroix, R., Arnaud, L., Giraudo, L., Hauchard, A., Sampol, J. and Dignat-George, F. (2009) Standardization of platelet-derived microparticle counting using calibrated beads and a Cytomics C500 routine flow cytometer: a first step towards multicenter studies? J. Thromb. Haemostasis **7**, 190–197

10 Orozco, A.F. and Lewis, D.E. (2010) Flow cytometric analysis of circulating microparticles in plasma. Cytometry, Part A **77**, 502–514

11 Lacroix, R., Judicone, C., Poncelet, P., Robert, S., Arnaud, L., Sampol, J. and Dignat-George, F. (2012) Impact of pre-analytical parameters on the measurement of circulating microparticles: towards standardization of protocol. J. Thromb. Haemostasis **10**, 437–446

12 Huica, R., Huica, S. and Moldoveanu, E. (2011) Flow cytometric assessment of circulating microparticles: towards a more objective analysis. Rom. Biotechnol. Lett. **16**, 6271–6277

13 Ogura, H., Kawasaki, T., Tanaka, H., Koh, T., Tanaka, R., Ozeki, Y., Hosotsubo, H., Kuwagata, Y., Shimazu, T. and Sugimoto, H. (2001) Activated platelets enhance microparticle formation and platelet–leukocyte interaction in severe trauma and sepsis. J. Trauma **50**, 801–809

14 Baj-Krzyworzeka, M., Szatanek, R., Weglarczyk, K., Baran, J., Urbanowicz, B., Branski, P., Ratajczak, M.Z. and Zembala, M. (2005) Tumour-derived microvesicles carry several surface determinants and mRNA of tumour cells and transfer some of these determinants to monocytes. Cancer Immunol. Immun. **55**, 808–818

15 Janša, R., Šuštar, V., Frank, M., Sušanj, P., Bešter, J., Manček-Keber, M., Kržan, M. and Iglič, A. (2008) Number of microvesicles in peripheral blood and ability of plasma to induce adhesion between phospholipid membranes in 19 patients with gastrointestinal diseases. Blood Cells Mol. Dis. **41**, 124–132

16 Rabinowits, G., Gerçel-Taylor, C., Day, J.M., Taylor, D.D. and Kloecker, G.H. (2009) Exosomal microRNA: a diagnostic marker for lung cancer. Clin. Lung Cancer **10**, 42–46

17 Lee, T.H., D'Asti, E., Magnus, N., Al-Nedawi, K., Meehan, B. and Rak, J. (2011) Microvesicles as mediators of intercellular communication in cancer-the emerging science of cellular 'debris'. Semin. Immunopathol. **33**, 455–467

18 Šuštar, V., Bedina-Zavec, A., Štukelj, R., Frank, M., Bobojević, G., Janša, R., Ogorevc, E., Kruljc, P., Mam, K., Šimunič, B. et al. (2011) Nanoparticles isolated from blood: a reflection of vesiculability of blood cells during the isolation process. Int. J. Nanomed. **6**, 2737–2748

19 Rubin, B.P. and Debnath, J. (2010) Therapeutic implications of autophagy-mediated cell survival in gastrointestinal stromal tumor after treatment with imatinib mesylate. Autophagy **6**, 1190–1191

20 Sanford, M. and Scott, L.J. (2010) Imatinib as adjuvant therapy for gastrointestinal stromal tumour. Drugs **70**, 1963–1972

21 Deininger, M., Buchdunger, E. and Druker, B.J. (2005) The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood **105**, 2640–2653

22 Savage, D.G. and Antman, K.H. (2002) Imatinib mesylate: a new oral targeted therapy. N. Engl. J. Med. **346**, 683–693
23 Scheijen, B. and Griffin, J.D. (2002) Tyrosine kinase oncogenes in normal hematopoiesis and hematological disease. Oncogene **21**, 3314–3333
24 Lassau, N., Lamuraglia, M., Chami, L., Leclère, J., Bonvalot, S., Terrier, P., Roche, A. and Le Cesne, A. (2006) Gastrointestinal stromal tumors treated with imatinib: monitoring response with contrast-enhanced sonography. AJR, Am. J. Roentgenol. **187**, 1267–1273
25 Demetri, G.D., von Mehren, M., Blanke, C.D., Van den Abbeele, A.D., Eisenberg, B., Roberts, P.J., Heinrich, M.C., Tuveson, D.A., Singer, S., Janicek, M. et al. (2002) Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N. Engl. J. Med. **347**, 472–480
26 Chen, M.Y., Bechtold, R.E. and Savage, P.D. (2002) Cystic changes in hepatic metastases from gastrointestinal stromal tumors (GISTs) treated with Gleevec (imatinib mesylate). AJR, Am. J. Roentgenol. **179**, 1059–1062
27 Diamant, M., Nieuwland, R., Pablo, R.F., Sturk, A., Smit, J.W. and Radder, J.K. (2002) Elevated numbers of tissue-factor exposing microparticles correlate with components of the metabolic syndrome in uncomplicated type 2 diabetes mellitus. Circulation **106**, 2442–2447
28 Siddiqui, M.A.A. and Scott, L.J. (2007) Imatinib. A review of its use in the management of gastrointestinal stromal tumors. Drugs **67**, 805–820
29 Ertmer, A., Huber, V., Gilch, S., Yoshimori, T., Erfle, V., Duyster, J., Elsaesser, H.P. and Schaetzl, H.M. (2007) The anticancer drug imatinib induces cellular autophagy. Leukemia **21**, 936–942
30 Miselli, F., Negri, T., Gronchi, A., Losa, M., Conca, E., Brich, S., Fumagalli, E., Fiore, M., Casali, P.G., Pierotti, M.A. et al. (2008) Is autophagy rather than apoptosis the regression driver in imatinib-treated gastrointestinal stromal tumors? Transl. Oncol. **1**, 177–186
31 McAucliffe, J.C., Hunt, K.K., Lazar, A.J.F., Choi, H., Qiao, W., Thall, P., Pollock, R.E., Benjamin, R.S. and Trent, J.C. (2008) A randomized, Phase II study of preoperative plus postoperative imatinib in GIST: evidence of rapid radiographic response and temporal induction of tumor cell apoptosis. Ann. Surg. Oncol. **16**, 910–919
32 Trent, C.J., Choi, H., Hunt, K., Macapinlac, H., McConkey, D., Charnsangravej, C., Abbruzzese, J., Benjamin, R.S. and Davis, B.D. (2005) Apoptotic and anti-vascular activity of imatinib in GIST patients. J. Clin. Oncol. **23** (Suppl.), 9001
33 Liu, Y., Tseng, M., Perdreau, S.A., Rossi, F., Antonescu, C., Besmer, P., Fletcher, J.A., Duensing, S. and Duensing, A. (2007) Histone H2AX is a mediator of gastrointestinal stromal tumor (GIST) cell apoptosis following treatment with imatinib mesylate. Cancer Res. **67**, 2685–2692
34 Akkerman, J.W. and De Bono, B. (2011) Systems biology to study platelet-related bleeding disorders. In Platelet Proteomics: Principles, Analysis and Applications (Garcia, A. and Senis, Y.A., eds), pp. 285–320, Wiley & Sons Inc., Hoboken
35 Liu, Y., Perdreau, S.A., Chatterjee, P., Wang, L., Kuan, S.-F. and Duensing, A. (2008) Imatinib mesylate induces quiescence in gastrointestinal stromal tumor cells through the CDH1–SKP2–p27^{Kip1} signaling axis. Cancer Res. **68**, 9015–9028
36 Liu, Y., Parry, J.A., Chin, A., Duensing, S. and Duensing, A. (2008) Soluble histone H2AX is induced by DNA replication stress and sensitizes cells to undergo apoptosis. Mol. Cancer **7**, 61
37 Mrówczyńska, L., Salzer, U., Iglič, A. and Hägerstrand, H. (2011) Curvature factor and membrane solubilisation, with particular reference to membrane rafts. Cell Biol. Int. **35**, 991–995
38 Kralj-Iglič, V. (2012) Stability of membranous nanostructures: a possible key mechanism in cancer progression. Int. J. Nanomed. **7**, 3579–3596
39 Urbanija, J., Tomšič, N., Lokar, M., Ambrožič, A., Čučnik, S., Rozman, B., Kandušer, M., Iglič, A. and Kralj-Iglič, V. (2007) Coalescence of phospholipid membranes as a possible origin of anticoagulant effect of serum proteins. Chem. Phys. Lipids **150**, 49–57
40 Frank, M., Manček-Keber, M., Kržan, M., Sodin-Šemrl, S., Jerala, R., Iglič, A., Rozman, B. and Kralj-Iglič, V. (2008) Prevention of microvesiculation by adhesion of buds to the mother cell membrane: a possible anticoagulant effect of healthy donor plasma. Autoimmun. Rev. **7**, 240–245
41 Šuštar, V., Janša, R., Frank, M., Hägerstrand, H., Kržan, M., Iglič, A. and Kralj-Iglič, V. (2009) Suppression of membrane microvesiculation: a possible anticoagulant and anti-tumor progression effect of heparin. Blood Cells, Mol., Dis. **42**, 223–227
42 Urbanija, J., Bohinc, K., Bellen, A., Maset, S., Iglič, A., Kralj-Iglič, V. and Kumar, P.B. (2008) Attraction between negatively charged surfaces mediated by spherical counterions with quadrupolar charge distribution. J. Chem. Phys. **129**, 105101
43 May, S., Iglič, A., Reščič, J., Maset, S. and Bohinc, K. (2008) Bridging like-charged macroions through long divalent rod-like ions. J. Phys. Chem. B **112**, 1685–1692
44 Iglič, A., Gongadze, E. and Bohinc, K. (2010) Excluded volume effect and orientational ordering near charged surface in solution of ions and Langevin dipoles. Bioelectrochemistry **79**, 223–227
45 Müller, I., Klocke, A., Alex, M., Kotzsch, M., Luther, T., Morgenstern, E., Zieseniss, S., Zahler, S., Preissner, K. and Engelmann, B. (2003) Intravascular tissue factor initiates coagulation via circulating microvesicles and platelets. FASEB J. **17**, 476–478
46 Morel, O., Morel, N., Freyssinet, J.-M. and Toti, F. (2008) Platelet microparticles and vascular cells interactions: a checkpoint between the haemostatic and thrombotic responses. Platelets **19**, 9–23
47 Melichar, B., Laco, J., Slovacek, L., Grossmann, P. and Vanecek, T. (2006) Fatal venous thromboembolism complicating imatinib therapy in a patient with metastatic gastrointestinal stromal tumor. J. Exp. Clin. Cancer Res. **25**, 607–610
48 Tetik, S., Demiralp, E.E. and Yardimici, K.T. (2005) Effect of imatinib mesylate on platelet aggregation and fibrinogen binding to isolated platelets. Adv. Mol. Med. **1**, 165–170

Received 18 September 2012
doi:10.1042/BST20120247