A Long-term Prospective Population Pharmacokinetic Study on Imatinib Plasma Concentrations in GIST Patients

Karel Eechoute\textsuperscript{1}, Martin N. Fransson\textsuperscript{3,4}, An K. Reyners\textsuperscript{2}, Floris A. de Jong\textsuperscript{1}, Alex Sparreboom\textsuperscript{1,5}, Winette T.A. van der Graaf\textsuperscript{2}, Lena E. Friberg\textsuperscript{3}, Gaia Schiavon\textsuperscript{1}, Erik A.C. Wiemer\textsuperscript{1}, Jaap Verweij\textsuperscript{1}, Walter J. Loos\textsuperscript{1}, Ron H.J. Mathijssen\textsuperscript{1}, and Ugo De Giorgi\textsuperscript{6}

Abstract

\textbf{Purpose:} Imatinib minimal (trough) plasma concentrations after one month of treatment have shown a significant association with clinical benefit in patients with gastrointestinal stromal tumors (GIST). Considering that a retrospective pharmacokinetic analysis has also suggested that imatinib clearance increases over time in patients with soft tissue sarcoma and GIST, the primary aim of this study was to assess systemic exposure to imatinib at multiple time points in a long-term prospective population pharmacokinetic study. As imatinib is mainly metabolized in the liver, our secondary aim was to elucidate the potential effects of the volume of liver metastases on exposure to imatinib.

\textbf{Experimental Design:} Full pharmacokinetic blood sampling was conducted in 50 patients with GIST on the first day of imatinib treatment, and after one, six, and 12 months. In addition, on day 14, and monthly during imatinib treatment, trough samples were taken. Pharmacokinetic analysis was conducted using a compartmental model. Volume of liver metastases was assessed by computed tomographic (CT) imaging.

\textbf{Results:} After 90 days of treatment, a significant decrease in imatinib systemic exposure of 29.3\% compared with baseline was observed ($P < 0.01$). For every 100 cm\textsuperscript{3} increase of metastatic volume, a predicted decrease of 3.8\% in imatinib clearance was observed.

\textbf{Conclusions:} This is the first prospective pharmacokinetic study in patients with GIST, showing a significant decrease of approximately 30\% in imatinib exposure after long-term treatment. This means that future “trough level – clinical benefit” analyses should be time point specific. GIST liver involvement, however, has a marginal effect on imatinib clearance. \textit{Clin Cancer Res; 18(20); 1–8.} ©2012 AACR.
Translational Relevance
Although imatinib treatment has proven efficacy in gastrointestinal stromal tumors (GIST), ultimately, progression of disease occurs due to drug resistance. Several mechanisms of drug resistance exist, with the putative role of "pharmacokinetic resistance" still largely unexplored. Efflux transporters (i.e., the ATP-binding cassette transporters ABCB1 and ABCG2) and influx transporters (i.e., the organic cation transporters OCT1 and the organic anion transporting peptide OATP1A2) may play an important role in this type of resistance. An altered expression of these transporters may lead to an increased clearance of the drug and lowered plasma concentrations of imatinib over time. In this article, long-term pharmacokinetics in patients with GIST are studied prospectively to elucidate its role in pharmacokinetic resistance, leading to implications for future studies and treatment with imatinib.

Materials and Methods
Patients
Patients with histologically confirmed GIST were accrued at start of imatinib therapy for long-term imatinib pharmacokinetic assessment in 2 Dutch and 2 Italian medical centers. Patients treated with drugs known to show major processes were evaluated. As no intravenous data were available, the initial bioavailability (F) was set to 1, meaning that clearance and volume of distribution (V) should be interpreted as the clearance (CL/F) and apparent volume of distribution (V/F), respectively.

The population pharmacokinetic analysis was conducted using nonlinear mixed effects modelling in the NONMEM software (version 7.1.2, ICON Development Solutions). The ADVAN5 subroutine combined with the first order conditional estimation method with interaction between random effects was used in the model building procedure. Perl-speaks NONMEM (version 3.2.12, http://psn.sourceforge.net/) and the R-package Xpose (version 4.3.0, http://xpose.sourceforge.net/) were used to automate model runs and for graphical analysis.

Presence of liver metastases was used as a dichotomous covariate (LIV) in the analysis. Time dependency (TIME), body weight (WT), volume of liver metastasis (LIVM), and liver metastatic volume proportional to liver volume (LIVR) were evaluated as continuous covariates. Also the medical center, in which patients were treated (CENTER), was analyzed as a covariate. As the number of patients from the 2 Italian centers was small, those 2 centers were grouped as one. Potential CYP-interactive drugs were highly restricted in this study. Hence, as major interactions were not expected, comedication was not included as a covariate. Recorded imatinib doses were used for modelling and when information was missing, it was assumed there was no change in the dosing until the next recorded dose. As only a minority of drug activity is attributable to CGP74588, this metabolite was not included in the model.

Interindividual variability (IIV) for an individual (ind) was modeled exponentially, and the residual errors for observed concentrations (\( e_{\text{obs}} \)) versus predicted...
Concentrations ($c_{\text{pred}}$) on the log scale were modelled using an additive error.

The NONMEM objective function value (OFV), which is proportional to $-2 \times \log$ likelihood of the data, was used to evaluate different model structures. A difference in OFV of at least 6.63 (corresponding to $P < 0.01$) was used to discriminate between competing models. NONMEM SEs were complemented with asymmetric confidence intervals by log-likelihood profiling (LLP) and a bootstrap ($N = 1,000$; stratified on LIV; http://psn.sourceforge.net/). A visual predictive check (VPC) was done to evaluate the predictive performance of the model. The observed data was overlaid with a 90% prediction interval based on 1,000 simulated data sets from the final model. The VPC was stratified on months of each 24-hour pharmacokinetic simulation in the following way: group 0 (day 1), group 1 (month 1, 2, and 3), group 2 (month 5 and 6), and group 3 (months $\geq$ 11).

In addition, to assess imatinib metabolic ratios ($\text{AUC}_{\text{tot}}$/CGP74588/$\text{AUC}_{\text{imatinib}}$) at start of therapy and after 6 and 12 months of treatment, a noncompartmental analysis of our steady-state imatinib and CGP74588 pharmacokinetic data after 1, 6, and 12 months was conducted, using WinNonlin software (Phoenix WinNonlin version 6.1, Pharsight Corporation).

**Computed tomographic-guided volumetric assessment of liver metastases**

Original Digital Imaging and Communications in Medicine (DICOM) files were imported in the open source OsiriX Imaging Software for MacOS X (OsiriX Foundation). All available computed tomographic (CT) scans at baseline, 6, and 12 months of treatment were studied (15). Two regions of interest (ROI) were assessed per CT slice: total liver area and liver metastasis area. Total liver areas were manually outlined by using the closed polygon selection tool and liver metastases lesions were drawn with the pencil tool, which allows a more precise drawing (Appendix A; ref. 15). Volumes of all 3-dimensional structures were then automatically calculated (Appendix A; ref. 15). Volumes of all 3-dimensional structures were then calculated on the basis of the above described volumetric assessments.

**Results**

**Patients**

Twenty-nine male and 21 female patients were included in this population pharmacokinetic analysis. Median follow-up period was 366 days (range, 59–761 days); 26 patients (13 males, 13 females) had evaluable liver metastases. Median volume of liver metastases was 5.8 cm$^3$ (range, 0.68–1,800 cm$^3$) and the median metastasis/liver volume ratio was 0.42% (range, 0.042%–61%). See Table 1 for clinical characteristics.

**Imatinib pharmacokinetics: time dependency**

A 2-compartment model with linear elimination and 5 transit compartments to describe the absorption process (Fig. 1) best fitted observed imatinib plasma concentrations. In addition, the relative $F$ and absorption rate ($k_a$) were both found to be significantly ($\Delta$OFV = 246.4) time-dependent according to:

$$F = 1 + \theta_F \times \exp(-\lambda \times \text{TIME}/24)$$

$$k_a,\text{ind} = (1 + \theta_{k_a} \times \exp(-\lambda \times \text{TIME}/24)) \times \begin{align*}
    k_a,\text{pop} \times \exp(\eta k_a,\text{ind})
\end{align*}$$

where TIME is the time in hours from first dose administration and $\lambda$ the decay constant, which was found to have
the same value for both parameters, \( \theta_f \) and \( \theta_{k_a} \) were more than 0 indicating that \( F \) and \( k_a \) start at a value of 1 + \( \theta_f \) and \( k_{a,\text{pop}} \times (1 + \theta_{k_a}) \), respectively, and both decrease with time to values of 1 and \( k_{a,\text{pop}} \), respectively. Time dependence on \( F \) and \( k_a \) could be replaced with time dependence on CL and \( k_s \) (increasing CL with time) with an almost equally good fit (\( \Delta \text{OFV} = +4.3 \)). However, this model was not robust and it was highly sensitive to initial parameter estimates, and quantitative testing of covariate effects could not be conducted. The predicted decreases in \( F \) and \( k_a \) as function of time after treatment start are shown in Fig. 2. After 30 days of treatment, \( F \) decreased with 17.4%, and after 90 days of treatment with 29.3%, compared with baseline. After 6 months and after 1 year, \( F \) decreased slowly further, with 32.2% and 32.5% compared with baseline (Fig. 2).

Noncompartmental analysis of our steady-state imatinib and CGP74588 pharmacokinetic data after 1, 6, and 12 months showed that the metabolic ratio (\( \text{AUC}_{\text{tau}} \text{CGP74588} / \text{AUC}_{\text{tau}} \text{imatinib} \)) remained stable over time (mean metabolic ratios ± SD at 1, 6, and 12 months: 0.72 ± 0.12; 0.75 ± 0.11; 0.74 ± 0.11, respectively).

**Imatinib pharmacokinetics: liver metastasis dependency**

Covariates WT, LIV, LIVM, and LIVR were tested on CL, \( V_{\text{Central}} \), and \( F \). CENTER were tested on CL, \( F \), and \( k_a \). LIVM on CL gave the largest drop in OFV (\( \Delta \text{OFV} = -21.8 \)), whereas the dichotomous covariate LIV was not significantly correlated with CL (\( \Delta \text{OFV} = -0.4 \)). The effect of LIVM could not be associated with the parameters \( \beta_f \) or \( \lambda \), governing time dependence on \( F \). Combining the effect of LIVM in CL with an effect of LIVM also in \( F \) was not significant (\( \Delta \text{OFV} = 0.0 \)). LIVR on CL was also significant (\( \Delta \text{OFV} = -11.0 \)) but was not significant (\( \Delta \text{OFV} = -2.3 \)) when combined with LIVM. Although including LIVM reduced the proportional residual error \( \epsilon \) from 35.4% to 35.0%, it did not reduce the IV in CL.

WT on CL gave a significant drop in OFV (\( \Delta \text{OFV} = -13.9 \)), but was omitted in the final model because of too much data imputation. CENTER had no significant effect on CL (\( \Delta \text{OFV} = -0.09 \)), \( F \) (\( \Delta \text{OFV} = -0.97 \)) or \( k_a \) (\( \Delta \text{OFV} = -3.74 \)) for 2 additional degrees of freedom, in which a difference in OFV by at least 5.99 corresponds with a significance level of \( P < 0.05 \).

The estimates of the final population pharmacokinetic model comprising the effect of LIVM on CL are presented in Table 2 together with NONMEM relative SEs (RSE), 95% confidence interval (CI) from the LLP and the median and 2.5 to 97.5 percentiles from 993 bootstrap replicates. The predicted decrease in CL as function of LIVM is expressed as:

\[
\text{CL}_{\text{POP}} = 9.12 \times (1 - 0.000381 \times \text{LIVM})
\]

This means that for every 100 cm\(^3\) increase in metastasis volume CL is decreased by 3.81%. The effect on apparent oral CL for the minimum (0.68 cm\(^3\)), median (5.8 cm\(^3\)), and maximum (1,800 cm\(^3\)) metastasis volume of the study population is 9.12 L/h, 9.10 L/h, and 2.87 L/h, respectively.

**Effects of pharmacokinetics on clinical outcome**

As shown in Appendix B, no statistically significant effects of pharmacokinetics on progression-free survival were found in the subset of patients treated with imatinib in an incurable setting. As this study was not
Table 2. Parameter estimates for the final population pharmacokinetic model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSEa (%)</th>
<th>Log-likelihood profiling</th>
<th>Estimates based on 993 bootstrap replicates stratified on LIV = 0/1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>95% CI</td>
<td>Median</td>
</tr>
<tr>
<td>CL (L/h)</td>
<td>9.12</td>
<td>7.5</td>
<td>7.94–10.5</td>
<td>9.13</td>
</tr>
<tr>
<td>V_{central} (L)</td>
<td>128</td>
<td>18</td>
<td>95.8–165</td>
<td>130</td>
</tr>
<tr>
<td>Q (L/h)</td>
<td>24.9</td>
<td>13</td>
<td>19.0–31.9</td>
<td>25.3</td>
</tr>
<tr>
<td>V_{periph} (L)</td>
<td>197</td>
<td>15</td>
<td>145–265</td>
<td>202</td>
</tr>
<tr>
<td>k_{a} (1/h)</td>
<td>0.699</td>
<td>20</td>
<td>0.510–0.942</td>
<td>0.710</td>
</tr>
<tr>
<td>k_{p} (1/h)</td>
<td>15.8</td>
<td>17</td>
<td>10.9–24.9</td>
<td>15.4</td>
</tr>
<tr>
<td>Residual error, ( \epsilon ) (%)</td>
<td>35.0</td>
<td>6.4</td>
<td>33.6–36.5</td>
<td>34.5</td>
</tr>
<tr>
<td>( \theta_f )</td>
<td>0.482</td>
<td>26</td>
<td>0.367–0.610</td>
<td>0.511</td>
</tr>
<tr>
<td>( \theta_{ka} )</td>
<td>1.18</td>
<td>28</td>
<td>0.72–1.82</td>
<td>1.15</td>
</tr>
<tr>
<td>( \lambda ) (1/day)</td>
<td>0.0256</td>
<td>37</td>
<td>0.0181–0.0357</td>
<td>0.0258</td>
</tr>
<tr>
<td>( \theta_{LVM} ) (1/cm³)</td>
<td>0.000381</td>
<td>3.9</td>
<td>0.000290–0.000440</td>
<td>0.000383</td>
</tr>
<tr>
<td>IIV CL (CV%)b</td>
<td>49.5</td>
<td>26</td>
<td>39.6–64.3</td>
<td>49.0</td>
</tr>
<tr>
<td>IIV V_{central} – k_{a} (CV%)b</td>
<td>70.9</td>
<td>26</td>
<td>50.7–104</td>
<td>70.4</td>
</tr>
<tr>
<td>IIV K_{p} (CV%)b</td>
<td>160</td>
<td>25</td>
<td>104–314</td>
<td>153</td>
</tr>
<tr>
<td>IIV V_{periph} (CV%)b</td>
<td>65.9</td>
<td>33</td>
<td>39.9–107</td>
<td>67.1</td>
</tr>
</tbody>
</table>

Abbreviations: LIV, dichotomous covariate indicating presence or absence of liver metastasis; CL, apparent oral clearance; V_{central}, volume of distribution for the central compartment; Q, intercompartmental clearance; V_{periph}, volume of distribution for the peripheral compartment; k_{a}, absorption rate constant; k_{p}, transit rate constant between the transit compartments; \( \theta_f \), change in bioavailability relative start of treatment; \( \theta_{ka} \), change in absorption rate relative start of treatment; \( \lambda \), decay constant; \( \theta_{LVM} \), variability based on liver metastatic volume; IIV CL, interindividual variability in apparent oral clearance; IIV V_{central}–k_{a}, interindividual variability in volume of distribution for the central compartment and absorption rate constant; IIV K_{p}, interindividual variability in the transit rate constant between compartments; IIV V_{periph}, interindividual variability in volume of distribution of the peripheral compartment.

aRelative Standard Error given by NONMEM
bCoefficient of Variation, calculated as (exp(\( \sigma_f^2 \)) – 1)0.5

\( \sigma_f^2 \) is the variance of the effect of the covariate on the CL.

Follow-up pharmacokinetic observations was several times larger than in previous retrospective imatinib population pharmacokinetic studies in patients with GIST, expressing the validity of the current data (11, 16). A multicompartmental pharmacokinetic model was built by use of this pharmacokinetic data set, showing a significant downward trend in systemic exposure to imatinib over time. From start of therapy up to 90 days, the initial imatinib exposure is reduced by approximately one third. From this time point on, the curve flattens, suggesting a further steady imatinib pharmacokinetics. Previous retrospective associations between imatinib trough levels at day 29 and clinical outcome benefit in patients with GIST thus need to be put into this perspective (10). As the predicted decrease in imatinib exposure at this time point is approximately 17%, the distribution of patients in groups based on imatinib trough levels will differ from the distribution seen after 3 months, when predicted systemic exposure to imatinib has dropped by approximately 30%. Within this 90-day period, significance of correlations between pharmacokinetics-based groups and clinical benefit will fluctuate. However, provided the proposed clinically relevant imatinib threshold level of 1,100 ng/mL is accurate, a number of patients will experience a drop below the efficacious plasma.

designed to study outcome, it is impossible to draw hard conclusions on associations between pharmacokinetics and survival.

Validation of the final population pharmacokinetic model

NONMEM RSEs, LLP 95% CIs, and bootstrap 2.5 to 97.5 percentiles are in good agreement for most parameters, the notable exception being the covariate parameter \( \theta_{LVM} \) for which the bootstrap percentiles contain the 0-value, whereas this is not the case for the corresponding LLP CI.

Visual predictive checks based on 1,000 simulations are shown in Fig. 3. Observed imatinib plasma concentrations (in \( \mu \)mol/L) showed good agreement with the 95% CIs for the 5th, 50th, and 95th percentiles of the simulated predictions. Time after dose (in hours) was used as the independent variable.

Discussion

This is the first prospective population pharmacokinetic study in patients with GIST, analyzing imatinib pharmacokinetics over an extensive median follow-up period of one year. During this study period, the reached number of
level after day 29 of imatinib therapy and may thus be underdosed when trough levels are only assessed after the first month of therapy. In addition, a delayed dose escalation might potentially be valuable in patients dropping below this threshold level. On the other hand, the proposed threshold may only be a marker for the actual clinically relevant imatinib plasma concentration cutoff value, which is reached after 3 months. This is highly relevant if therapeutic drug monitoring should be applied in future imatinib dosing. Imatinib plasma level monitoring in patients with GIST should therefore be time point-specific and repeated after the first quarter of the first year of imatinib treatment. This will have to be taken into account when designing randomized studies aimed to validate the use of imatinib plasma level monitoring in patients with GIST in a recent retrospective pharmacokinetic analysis, as the median time from start of therapy to first pharmacokinetic assessment in this study was 5.5 months (16). In light of our findings, a major drop in imatinib plasma levels has already occurred during this lag time.

Currently, literature on mechanisms that may drive these acquired pharmacokinetic phenomena in imatinib-treated patients is scarce (17). Visually, maximum imatinib plasma concentrations in our study population ($C_{\text{max}}$) are lower and time to reach $C_{\text{max}}$ is longer after 3 months of dosing (Fig. 3) as compared with the first 3 months of treatment. In addition, the final pharmacokinetic model fitted significantly better when adding a time dependence in absorption rate as a covariate ($\text{OFV} = –42.9$). These observations suggest that the observed time-dependent drop in imatinib exposure is located at the absorption phase. Hence, there may be a change in activity or expression of drug transporters involved in facilitated or active transport of imatinib. However, drug uptake and efflux transporters have shown a limited effect on imatinib absorption and excretion (17, 18), and $\text{in vivo}$ data showed no upregulation of drug efflux transporters after long-term treatment with imatinib (19). So, up until now, key mediators of imatinib transport during absorption and elimination have not been identified. On the other hand, as imatinib is extensively metabolized by CYP3A4 to its main metabolite CGP74588 (13), upregulation of liver enzymatic function may also (in part)
account for the observed drop in imatinib plasma levels. This pharmacokinetic mechanism would possibly have a minor impact on imatinib efficacy as CGP74588 is equipotent to its parent compound and has a longer terminal elimination half-life (20). However, metabolic ratios (AUC\textsubscript{tau} CGP74588 / AUC\textsubscript{tau} imatinib) remained stable during the first year of treatment, implying that upregulation of metabolic activity does not occur over time. Finally, increasing patient nonadherence to imatinib treatment over time may also be involved in the observed decline in systemic exposure. This is less likely to be of large influence on exposure though, as full pharmacokinetic time-profiles show limited accumulation (a limited effect of single imatinib trough levels on total exposure). Occasional dosing delays will therefore have a limited effect on imatinib exposure. Moreover, an observational study evaluating compliance in 28 imatinib-treated GIST patients at 2 time points, detected no significant difference in nonadherence rates after 90 days of imatinib use (21).

Secondary objective of this study was to evaluate if volume of liver metastasis in patients with GIST is predictive for imatinib exposure. Liver metastasis volume seemed to have a minor effect on imatinib CL/F, rendering some clinical significance with massive liver involvement, as for every 100 cm\textsuperscript{3} increase of metastatic volume, a predicted decrease of approximately 4% in CL/F is observed. This is in concordance with an earlier phase I side study that reported limited effect of liver dysfunction on imatinib exposure (22). These data together with our present results indicate that neither liver metastatic involvement nor routine liver function testing highly correlate with hepatic CYP activity in imatinib-treated patients.

To conclude, this observational population pharmacokinetic study shows that imatinib pharmacokinetics in patients with GIST stabilizes after approximately 3 months of dosing with a significant decrease in systemic exposure of approximately 30% compared with baseline, most likely due to reduced absorption. This means that future "trough level to clinical benefit" analyses should be time point-specific and need to incorporate relevant tumor biology and patient characteristics in multivariate analyses. Such survival analyses based on imatinib pharmacokinetics should be conducted in large (multicenter) patient populations and could, ultimately, lead to therapeutic fine tuning in which a minimal effective imatinib dose for an individual patient can be defined on accurate time points in a treatment course.

Disclosure of Potential Conflicts of Interest
A.K.L. Reyners and W.T.A. van der Graaf are consultant/advisory board members of Novartis and W.T.A. van der Graaf has a commercial research grant from Novartis. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: K. Eechoute, F.A de Jong, W.T.A. van der Graaf, J. Verweij, R.H.J. Mathijssen, U. De Giorgi

Development of methodology: K. Eechoute, F.A de Jong, J. Verweij, W.J. Loos, R.H.J. Mathijssen


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Eechoute, A.K.L. Reyners, F.A de Jong, E.A.C. Wiemer


Model development: M.N. Fransson, L.E. Friberg

Acknowledgments
The authors thank Maurizio Marangolo, Paolo Casali, Alessandro Ruggero, Giammaria Fiorentini, Ryan M. Franke, Peter de Bruijn, Inge M. Ghiobadi Moghaddam-Helmantel, Bernadette Vertogen, and Gina Turini for their involvement in this study.

Grant Support
This work was supported by the EC FP6 CONTICANET network of excellence (LSHC-CT-2005-018806) from the European Commission and by the Swedish Knowledge Foundation through the Industrial PhD Programme in Medical Bioinformatics.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 13, 2012; revised July 4, 2012; accepted July 14, 2012; published OnlineFirst July 31, 2012.

References
4. van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S, et al. Safety and efficacy of imatinib (STI571) in meta-

Clinical Cancer Research

A Long-term Prospective Population Pharmacokinetic Study on Imatinib Plasma Concentrations in GIST Patients

Karel Eechoute, Martin N. Fransson, An K. Reyners, et al.

Clin Cancer Res  Published OnlineFirst July 31, 2012.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-12-0490

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2012/07/30/1078-0432.CCR-12-0490.DC1
http://clincancerres.aacrjournals.org/content/suppl/2012/10/11/1078-0432.CCR-12-0490.DC2

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.