Imatinib – an overview

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DEININGER MWN
Oregon Health & Science University Center for Hematologic Malignancies, Portland, OR, USA

Historical perspective and chemical development

The potential of Abl kinase inhibitors for the treatment of Abl-related leukemias was suggested as early as 1989.[1] Several tyrphostins, a series of compounds derived from the erbstatin, an inhibitor of the epidermal growth factor receptor were shown to be potent and rather specific inhibitors of Bcr-Abl, with biological activity in cell lines derived from patients with chronic myeloid leukemia (CML).[2] Tyrphostins were however not developed clinically. Imatinib, the first clinically viable Abl kinase inhibitor, is the result of a medicinal chemistry project initiated by Ciba Geigy (now Novartis) in the early 1990s, with protein kinase C as the initial target. During this project, a 2-phenylaminopyrimidine derivative was identified as the lead compound[3,4] (Figure 1).

This compound, a low potency inhibitor of both serine/threonine and tyrosine kinases, served as the starting point for the synthesis of a series of derivatives. The cellular activity (as opposed to activity in cell free systems) was enhanced by the addition of a 3'-pyridyl group at the 3'-position of the pyrimidine (Figure 1A).

Activity against tyrosine kinases was enhanced by introduction of a benzamide group at the phenyl ring (Figure 1B). Analysis of structure activity relationships showed that substitutions at the 6-position of the anilino phenyl ring led to loss of PKC inhibition, while the introduction of a *flag-methyl* group at this position retained or enhanced activity against tyrosine kinases, including Abl (Figure 1C). The first series of compounds had low water solubility and poor oral bioavailability. The attachment of a highly polar side chain, N-methylpiperazine, markedly improved solubility and oral bioavailability (Figure 1D). STI571 (formerly CGP57148B, now imatinib mesylate, Gleevec® or Glivec®) emerged as the most promising compound for clinical development in Philadelphia chromosome (Ph)-positive leukemia, since it had the highest selectivity for growth inhibition of Bcr-Abl expressing cells. Biochemical studies showed that imatinib acts as an ATP-competitive inhibitor of the Abl kinase.[5]

Structure function analysis

The crystal structure of the Abl kinase domain (KD) in complex with imatinib was solved only after the clinical efficacy of the drug was established beyond doubt.[6,7]

The catalytic domains of kinases have a canonical bilobar structure, with a smaller N-terminal predominantly consisting of antiparallel β-sheets and a larger, predominantly helical, C-terminal lobe. The amino acid residues critical for catalytic activity line the cleft between the two lobes. Important structural features include the ATP-binding loop (p-loop), the highly conserved aspartate-phenylalanine-glycine (DFG) motif and the activation loop. The position of the activation loop is central for regulation of kinase activity. Surprisingly, the position in the AblK: imatinib complex resembled the *closed* activation loop conformation of inactive kinases (Figure 2). In this conformation the activation loop of Abl occludes the mouth of the kinase, mimicking a substrate and preventing productive binding of substrate and ATP. In the *open* conformation the activation loops swings away from the catalytic center, with a short β sheet within the loop providing a scaffold for binding of substrates. While the conformations of the activation loops in active kinases are very similar to each other, they are much more distinct in inactive kinases. Thus, the finding that imatinib binds an inactive conformation of Abl explained the selectivity of imatinib for Abl over closely related kinases such as

Preclinical profiling

In vitro activity. The IC50 values for inhibition of platelet derived growth factor receptor (PDGFR), Kit and Abl in *in vitro* kinase assays are in the nanomolar range.

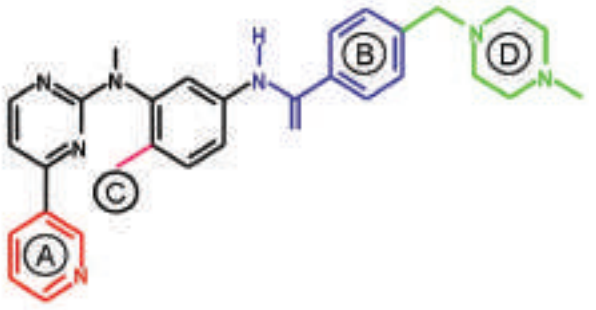

Figure 1. Lead optimization. Development of imatinib from a 2-phenylaminopyrimidine backbone (shown in black). (A) Activity in cellular assays was improved by introduction of a 3'pyridyl group (red) at the 3'- position of the pyrimidine. (B) Activity against tyrosine kinases was further enhanced by addition of a benzamide group (blue) to the phenyl ring. (C) Attachment of a *flag-methyl* group (magenta) ortho to the diaminophenyl ring strongly reduced activity against protein kinase C. (D) Addition of an N-methylpiperazine (green) increased water-solubility and oral bioavailability. *Adapted from Deininger et al. Blood 2005;105:2640-53.*

Some activity was seen against Lck, a Src kinase, whereas other tyrosine and serine/threonine kinase were resistant to concentrations >100 μM (Table 1). These data were mirrored in cellular phosphorylation and proliferation assays of cell lines expressing activating mutations of PDGFR, Kit and Abl, although the IC_{50} levels are approximately 10-fold higher, in the range of 0.25–0.5 μM.[8] Whether this reflects drug efflux, the presence of a cellular protein that that interferes with drug binding or simply the fact that intracellular ATP levels are higher than the concentrations used in kinase assays is unknown.

BCR-ABL-positive cells treated with imatinib undergo apoptosis, consistent with inhibition of apoptosis by the tyrosine kinase activity of Bcr-Abl. Colony formation by CML progenitor cells was inhibited at similar concentrations, with relatively little effect on normal progenitor cells at concentrations below one μM.[8]

Animal models. Imatinib administered twice daily by oral gavage was shown to dose-dependently inhibit the growth of tumors in mice injected subcutaneously with 32D cells expressing Bcr-Abl, while growth of tumors expressing active Src was not affected.[9] However, no tumor eradication was seen.

This may reflect the rather unsatisfactory pharmacokinetics of imatinib in mice, as a subsequent study in a nude mouse model using the human CML cell line Ku812 clearly demonstrated that continuous inhibition of Bcr-Abl activity requires three times daily dosing and that with this schedule a significant proportion of animals are cured.[10] Inadequate dosing may also be the reason why only moderate activity was seen in a murine CML model.[11]

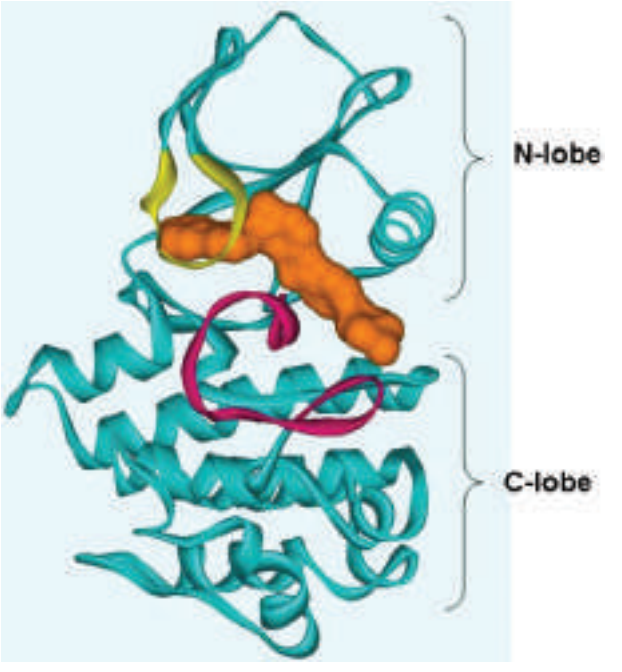

Figure 2. Structure of the Abl kinase domain in complex with imatinib. Shown is the conformation of Abl (blue) in complex with imatinib (orange), with the activation loop (magenta) in a *closed* conformation. The ATP binding loop (yellow) folds down over the inhibitor (induced fit). Figure prepared by Sandra W. Cowan-Jacob based on reported structures.[7,53] *Adapted from Deininger et al. Blood. 2005;105:2640-53.*

Clinical trials

Phase I studies. Phase I studies started in June 1998 and established the efficacy of imatinib in patients with CML in all phases. In patients with late chronic phase a complete hematologic response (CHR) was seen in close to 100% at doses of at least 300 mg imatinib daily, and a sizable fraction of patients attained major cytogenetic response (MCR) or complete cytogenetic responses (CCR).[12] Results in patients with blast crisis were less impressive, with high rates of relapse after initial responses, in particular with lymphoid blast crisis.[13] Non-hematologic toxicity was usually mild and included fluid retention, nausea, diarrhea, muscle cramps, arthralgia and skin rashes. Grade 3/4 neutropenia and thrombocytopenia were common, particularly in advanced disease. It is thought that rather than toxicity *sensu strictu* this reflects suppression of the leukemic clone in patients without sufficient reserve of Ph-negative progenitor cells to restore hematopoiesis. No maximum tolerated dose (MTD) was formally established in these trials. Studies in patients with gastrointestinal stromal tumors subsequently established the MTD as 1000 mg daily.[14] With 400 mg imatinib daily mean trough levels at steady state were 1.43 μM, approximately 5-fold the IC_{50} in cell proliferation assays. Based on this and almost 100% CHR with 300 mg imatinib daily in

Table 1. Inhibition of protein kinases by imatinib.

Enzyme	Substrate phosphorylation IC_{50} [μM]	Cellular tyrosine phosphorylation IC_{50} [μM]
c-ABL	0.2; 0.025*	ND
v-ABL	0.038	0.1-0.3
$p210_{BCR-ABL}$	0.025*	0.25
$p185_{BCR-ABL}$	0.025*	0.25
TEL-ABL	ND	0.35
PDGF-R α and β	0.38 (PDGF-Rβ)	0.1
Tel-PDGF-R	ND	0.15
c-KIT	0.41	0.1
FLT-3	>10	>10
Btk	>10	ND
c-FMS and v-FMS	ND	>10
c-SRC	>100	ND
v-SRC	ND	>10
c-LYN	>100	ND
c-FGR	>100	ND
LCK	9.0	ND
SYK (TPK-IIB)	>100	ND
JAK-2	>100*	>100
EGF-R	>100	>100
Insulin receptor	>10	>100
IGF-IR	>10	>100
FGF-R1	31.2	ND
VEGF-R2 (KDR)	10.7	ND
VEGF-R1 (FLT-1)	19.5	ND
VEGF-R3 (FLT-4)	5.7	ND
TIE-2 (TEK)	>50	ND
c-MET	>100	ND
PKA	>500	ND
PPK	>500	ND
PKCα, β1, β2, γ, δ, ε, ζ, or η	>100	ND
Protein kinase CK-1, CK-2	>100	ND
PKB	>10	ND
p38	>10	ND
PDK1	>10	ND
c-Raf-1	0.97	ND
CDC2/cyclin B	>100	ND

*Imatinib concentrations causing a 50% reduction in kinase activity (IC_{50}) are given. *IC_{50} was determined in immunocomplex assays. PDGF-R, platelet-derived growth factor receptor; Btk, Bruton's tyrosine kinase; TPK, tyrosine-protein kinase; EGF-R, epidermal growth factor receptor; IGF-IR, insulin-like growth factor receptor I, FGF-R1, fibroblast growth factor receptor 1; VEGF-R, vascular endothelial growth factor receptor; PKA, cAMP-dependent protein kinase; PPK, phosphorylase kinase; PKC, protein kinase C; CK, casein kinase; PKB, protein kinase B (also known as Akt); PKD1, 3-phosphoinoside-dependent protein kinase 1. ND – not done.*

Table 2. Responses to imatinib in blast crisis and accelerated phase of CML.

	Overall hematologic response/CHR	Sustained hematologic responses (> 4 weeks)	MCR	CCR	Median survival
Myeloid blast crisis (%) (n = 229)	52/15	31	16	7	6.8 months
Ph-positive ALL* (%) (n = 56)	59/22	27	not available	not available	4.9 months
Accelerated phase (%) (n = 181)	82/53	69	24	17	Not reached
Chronic phase after failure of IFN	95% sustained CHR		60	41	Not reached

** Also lymphoid blast crisis of CML. CHR – complete hematologic response; MCR – major cytogenetic response; CCR – complete cytogenetic response.*

Table 3. Responses to imatinib vs IFN plus cytarabine in newly diagnosed CML patients in chronic phase.

	CHR	MCR	CCR	Progression-free survival (14 months)
Imatinib (%) (n = 553)	95.3	85.2	73.8	92.1
IFN+ Cytarabine (%) (n = 553)	55.5	22.1	8.5	73.5
p=	0.001	0.001	0.001	0.001

Median duration of follow up = 19 months. CHR – complete hematologic response; MCR – major cytogenetic response; CCR – complete cytogenetic response

chronic phase patients a dose of 400 mg daily was chosen for the phase II studies.

Phase II studies. Several multicenter trials enrolled more than 1,000 patients worldwide and largely confirmed the phase I results (Table 2).[15-18] However, results for chronic phase patients were considerably better, with >40% of patients achieving CCR. For patients with advanced disease, the initial dose of 400 mg daily was subsequently increased to 600 mg, when safety data with the higher dose became available. Retrospective comparison of the two groups demonstrated superior overall and progression free survival for the 600 mg cohort. The combination of phase I and II studies led to regulatory approval of imatinib for the treatment of advanced CML and relapsed Ph-positive acute lymphoblastic leukemia (ALL).

Phase III study. Imatinib was then compared to the combination of interferon-α and low dose subcutaneous cytarabine in a phase III trial. This study allowed crossover between arms for intolerance and inadequate response. Imatinib was found to be vastly superior in terms of rates of CHR, MCR, CCR, and freedom from progression to accelerated phase and blast crisis (Table 3).[19] Overall survival was not significantly different at first, reflecting the fact that many patients in the interferon/cytarabine arm could be rescued with imatinib, but an advantage for imatinib was subsequently demonstrated with longer follow-up.[20] This study established imatinib as the first line drug therapy for newly diagnosed patients with CML.

Prognostic factors in newly diagnosed patients. High Sokal risk is correlated with a lower rate of CCR and shorter progression free survival in newly diagnosed patients.[19] However, time-dependent variables that essentially measure the *in vivo* drug sensitivity of the disease are more powerful predictors of outcome. Thus, patients with CCR who achieve a reduction of BCR-ABL transcripts by 3-log or more (termed a major molecular response, MMR) at 12 months have a lower risk of progression than patients with CCR but <3-log reduction of *BCR-ABL* mRNA, and the latter have a lower risk than patients without CCR at 12 months.[21]

High dose imatinib

High dose imatinib, i.e. a dose of 800 mg daily rather than 400 mg, were used in several single-armed and single institutional studies of patients with or newly diagnosed chronic phase. These studies generally show a trend towards more profound responses at earlier time points.[22,23] These data are consistent with the observation that that hematologic or cytogenetic refractoriness to standard dose imatinib can be overcome by dose escalation in approximately 1/3 of patients.[24] Whether high dose therapy is truly superior to standard dose remains to be proven in a prospective randomized study. Several large trials to this end are currently underway in Europe and the US.

Resistance to imatinib

Acquired or secondary resistance, defined as loss of a given level of response, must be distinguished from primary resistance or refractoriness, as the underlying mechanisms may be different. The key feature of acquired resistance is reactivation of Bcr-Abl kinase, as demonstrated by an increased phosphorylation of CrkL, a specific substrate of Bcr-Abl.[25] Mutations in the KD of Abl that impair imatinib were detected in 50-90% of patients with acquired resistance to imatinib.[25-32] BCR-ABL gene amplification or increased levels of BCR-ABL mRNA is present in a minority of patients.[32]

KD mutations. Resistance inducing point mutations of Bcr-Abl have been described in more than 20 different amino acids but tend to cluster in certain regions of the KD, most importantly the P-loop, threonine 315 and methionine 351 (Figure 3). KD mutations may confer imatinib resistance by one of three mechanisms. *(i)* Mutations may alter sites directly involved in drug binding. The best example is T315, which makes a hydrogen bond with imatinib. In the T315I mutant, threonine is replaced with the bulky isoleucine, eliminating the hydrogen bond and causing a sterical clash with imatinib. *(ii)* Mutations may prevent the conformational changes required for drug binding. Examples include mutations of the p-loop. *(iii)* Mutations may favor the active conformation of the kinase, from which imatinib is sterically

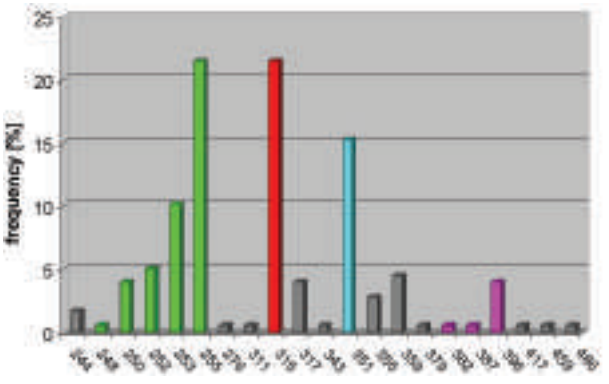

Figure 3. Frequency of BCR-ABL mutants isolated from clinical specimens (n=166). Mutations cluster in four distinct regions of the kinase domain. Mutations of the ATP-binding loop (amino acids 248-255) are most common, followed by mutations of T315, which forms a hydrogen bond with imatinib. M351 interacts with the SH2 domain and participates in autoregulation of kinase activity. The fourth cluster encompasses the activation loop (amino acids 379-398). *From Deininger et al. Blood. 2005;105: 2640-53.*

excluded. This mechanism probably underlies the resistance of activation loop mutants such as H396P. The rather common M351T mutant may also belong to this category, as it eliminates the site of an inhibitory interaction between the Abl kinase and SH2 domain, which may favor the kinase active conformation. The level of imatinib resistance conferred by these mutations is extremely variable. For example the E255K and 0T315I mutants are more than 100-fold less sensitive to imatinib in kinase assays, while the M351T mutant is only 3-4-fold less sensitive and the sensitivity of F311L is close to unmutated Bcr-Abl[33] (Figure 4). This has clinical implications, as a response to imatinib may be recaptured with dose escalation in the case of mutants with low resistance, whereas this would hardly be efficacious for highly resistant mutants such as T315I. Other treatment options for resistant disease include combinations with conventional agents such as cytarabine or other signal transduction inhibitors. However, given that Bcr-Abl kinase activity remains central to disease pathogenesis at the time of recurrence, novel more potent Abl kinase inhibitors such as dasatinib[34] or AMN107[35] with activity against most KD mutants except T315I are more promising. Clearly, patients who progress to accelerated phase or blast crisis should be offered an allogeneic transplant if eligible.

Disease persistence

The incidence of refractoriness is closely correlated with disease phase and the level of response considered. Thus, hematologic refractoriness is common in myeloid blast crisis but very rare in chronic phase, while refractoriness at the molecular level (i.e. failure to achieve negativity by RT-PCR) is the rule even in chronic phase (Figure 5). Since most patients, at least in developed countries, are diagnosed in chronic phase, this group of patients is clearly most relevant. There is currently no universally accepted definition of complete molecular response (CMR), but an emerging consensus is that the diagnosis of CMR should be based on a PCR test with a sensitivity of at least 1:105 (leukemia cells/normal cells), a level of sensitivity that is usually only achieved with nested PCR, and at least one negative confirmatory test.[36]

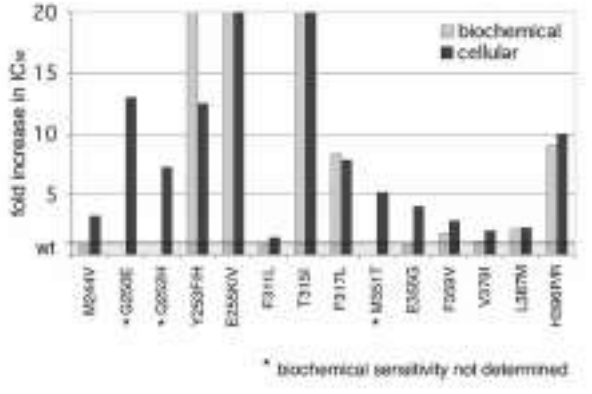

Figure 4. Relative sensitivity of selected Bcr-Abl kinase domain mutants in *in vitro* kinase and cell proliferation assays. *From Corbin et al. Blood. 2003;101(11):4611-4.*

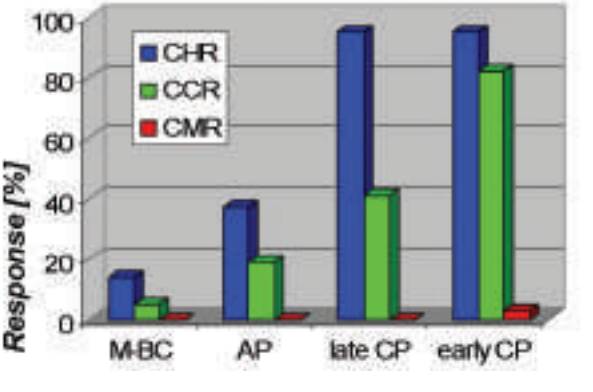

Figure 5. Frequency of complete hematologic, complete cytogenetic and complete molecular responses in CML patients treated with imatinib, according to disease phase. *From Deininger et al. Blood. 2005;105(7):2640-53.*

Table 4. Reported rates of complete molecular remission.

CMR (%)	Disease state	Imatinib dose (mg)	authors
3	CP1, up-front	400	Hughes et al. 2003[21]
0	IFN failure	400	Merx et al.2002[54]
13	IFN	400	Kantarjian et al.2003[55]
41	IFN	800	Cortes et al.2003[23]
28	CP1, up-front	800	Kantarjian et al.[22]

The lack of a universal consensus may contribute to the large variations in CMR incidence across published studies (Table 4). The fact that in patients with CCR disease recurrence is the rule after discontinuation of therapy except in patients previously treated with allogeneic stem cell transplantation indicates that the persistent leukemia cell population has full leukemogenic potential,[37-39] suggesting that patients remain at risk of relapse. It is currently unknown which properties allow leukemic stem cells to survive in the presence of imatinib. It is not even known whether Bcr-Abl kinase is active in the persistent cells or not and thus it is unknown whether disease persistence is Bcr-Abl-dependent or not. Several mechanisms have been proposed (reviewed in[40]). CML patients are known to harbor a population of primitive quiescent cells that have the capacity of repopulating immunodeficient mice.[41] *In vitro* these cells are resistant to imatinib,[42] and it has been suggested that they may be equivalent to the persistent population, a notion that is unproven. A small study reported KD mutations in 5/13 patients in CCR and mutations were detected in four additional patients on follow-up, associated with a rise in BCR-ABL mRNA.[43] Importantly, the majority of mutations detected in these patients confer only a moderate degree of imatinib resistance, suggesting that they may be sufficient to maintain the clone's viability in the presence of drug, but not support its expansion. As larger studies reported a much lower incidence of relapse in patients with CCR, this finding may not be relevant for the 'typical' patient in CCR. Imatinib is a substrate for drug transporters such as Pgp,[44-47] it is also possible that intracellular drug concentrations in the leukemic stem cells are much lower than plasma levels suggest. As imatinib is actively transported into the cells by Hoct1, another drug transporter, lack of this protein in leukemic stem cells could also be responsible.[48,49] Lastly, it has been reported that the levels of BCR-ABL mRNA are much higher in immature progenitor cells than in their differentiated progeny.[50] If this was correlated with high protein levels, then such cells would be innately capable of tolerating much higher drug concentrations. With all the aforementioned mechanisms Bcr-Abl is central to the persistent phenotype. This implies that more potent Abl inhibitors with activity against KD mutant Bcr-Abl that are not substrates for drug pumps may be able to eradicate persistent disease. However, it is also conceivable that leukemic stem cells are not or not entirely dependent on Bcr-Abl for their survival. In fact, CML progenitor cells are not completely growth factor independent,[51] suggesting that physiological survival pathways are intact and may be utilized if Bcr-Abl is inhibited. In line with this, activation of mitogen activated kinase signaling has been shown in $CD34^+$ cells treated with imatinib *ex vivo*.[52]

Summary

Imatinib is the standard drug therapy for CML patients in all phases. Non-hematologic side effects are usually mild. The durability and depths of response is inversely correlated with disease stage at initiation of therapy. Time-dependent variables such as CCR are powerful predictors of outcome. Resistance is almost invariably Bcr-Abl-dependent and caused by KD mutations and less frequently increased expression of Bcr-Abl. Alternative Abl kinase inhibitors are emerging as the most promising strategy to overcome resistance. Persistence of disease at the molecular level is the rule even in chronic phase patients, and recurrence is the rule after discontinuation of drug. Elucidating the underlying mechanisms may be critical to the long-term success of imatinib.

Acknowledgement

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