Abstract. In recent years, much has been discussed on the development of oral anticancer treatment in terms of practical aspects and convenience for the patient. Less has been devoted to the potential of subcutaneous administration as a parenteral alternative. However, recent approvals (bortezomib, omacetaxine, trastuzumab) seem to show a renewed interest in this route of administration. All anticancer agents given subcutaneously display a very high bioavailability (>80%) and are rapidly absorbed (except the monoclonal antibodies trastuzumab and alemtuzumab). Subcutaneous delivery does not impact on the rate of elimination when compared to the intravenous route (azacitidine, cladribine, bortezomib, trastuzumab). Some formulations may be self administered in educated patients (methotrexate, cladribine) but others require hospitalization (omacetaxine). When available, comparative studies with intravenous administration showed comparable clinical issues with an advantage for subcutaneous bortezomib with regard to the occurrence of peripheral neurotoxicity. Subcutaneous formulations of trastuzumab and, in the future rituximab, may allow for ambulatory treatment and self-administration. From an economic point of view, subcutaneous formulations of monoclonal antibodies may lead to lower healthcare costs but will have to face the arrival of less expensive intravenous biologically similar agents (‘biosimilars’) that will reduce the cost of hospitalization.

In recent years, much has been said on the development of oral anticancer treatment in terms of practical aspects and convenience for the patient when compared to intravenous administration. The major drawbacks of intravenous injection are the risks of bloodstream infection and the need for a hospital setting. As of January 2014, more than 60 oral anticancer agents were available and among them, 22 kinase inhibitors have been approved worldwide since 2001 (imatinib). Less has been devoted to the potential of subcutaneous administration as a parenteral alternative, probably due to the cytotoxic nature of these agents. However, recent approvals (azacitidine, bortezomib, omacetaxine, trastuzumab and in the near future, rituximab) has led to a re-newed interest in this route of administration. Besides luteinizing hormone-releasing hormone analogs (androgen deprivation therapy in prostate cancer, endocrine therapy in breast cancer) and cytokines (aldesleukin/interleukin-2 and interferon-alpha) in the treatment of renal carcinoma and melanoma, very few (n=8) anticancer agents are given subcutaneously. This is due to the fact that most of them are irritant or vesicant (i.e. known to cause local damage in the subcutaneous or subdermal tissues after inadvertent infiltration, also called extravasation) (1). Subcutaneous delivery, therefore, applies to non-vesicant agents. Currently approved anticancer drugs for subcutaneous injection are methotrexate, cytarabine, azacitidine, cladribine, bortezomib, omacetaxine, bleomycin and trastuzumab (Table I). Based on the official labeling, azacitidine (in Europe) and omacetaxine are only administered by subcutaneous injection, while the others may also be given intravenously or through other routes. A subcutaneous formulation of rituximab is under development as a parenteral alternative of the intravenous form. From a commercial perspective, formulations of trastuzumab and rituximab aim to counter the next arrival of intravenous biologically similar agents (generic versions of these high-priced therapeutic proteins and referred as to ‘biosimilars’). Off-label subcutaneous administration of alemtuzumab has also been reported. To the best of my knowledge, no general article has focused on the subcutaneous delivery of anticancer agents in humans. The goal of this short review is to present what is known of the use of subcutaneous anticancer agents in humans (animal data are excluded).
Subcutaneous Injection

Subcutaneous delivery is performed in the hypodermis (under the skin) generally as a short injection (few seconds or minutes). This route of administration is adapted to chronic treatments, constitutes an alternative for patients with poor venous access, limits infectious problems, may be performed in the ambulatory setting and allows self-administration in educated patients. It is more convenient for patients and medical staff, and requires less pharmaceutical preparation. In all, it may reduce costs for the healthcare system. However, the volume of injection has to be limited (1-5 ml) for pain reasons, necessitating the use of concentrated formulations and sometimes two separate sites of administration. For macromolecules (antibodies, see below), the subcutaneous formulation may require excipients that facilitate administration. In addition, the product needs to diffuse in the extracellular matrix to reach the blood (absorption phase). Even if the site of injection is very close to blood vessels, not all drugs are efficiently and systematically delivered (i.e. the absolute bioavailability may be less than 100% when compared to intravenous injection). Furthermore, a delay in absorption may occur and the time to the maximal effect (pharmacodynamic endpoint) may be longer when compared to the intravenous route. The extent and rate of absorption may also vary according to the anatomic region of subcutaneous injection (abdomen, upper arm, thigh) (2). Regarding specific side-effects, subcutaneous delivery may lead to injection-site reactions including erythema and pain and may be more immunogenic.

Anticancer Agents Given Subcutaneously

Methotrexate. Methotrexate is an antifolate agent used in the treatment of various types of cancers. It is primarily given by the intravenous route in oncology due to the high doses used (i.e. >1 g/m²). The oral route may be used but is limited by non-linear, incomplete and variable absorption (3). Consequently, oral as well as subcutaneous or intramuscular delivery of methotrexate is dedicated to
 intermittent, low doses (20-30 mg). These dosages are rarely used in oncology (maintenance of remission treatment in acute lymphoblastic leukemia) and mostly apply to the treatment of autoimmune diseases (rheumatoid arthritis).

Subcutaneous methotrexate was developed as an alternative to the oral route to circumvent the variability of absorption. When methotrexate (40 mg/m²) was injected subcutaneously in five children with acute lymphoblastic leukemia on maintenance therapy, it was found to be completely absorbed when compared to the intravenous dose (4). The concentration peaks (Cₘₐₓ) were comparable (7.4 μM versus 11.4 μM for the subcutaneous and intravenous routes, respectively) (Table II). The subcutaneous administration was well-tolerated in children (4). Since then, pre-filled syringes of methotrexate (50 mg/ml) have been commercialized, adding simplicity to the subcutaneous injection.

**Cytarabine.** Cytarabine, or cytosine arabinoside, is an analog of the pyrimidine nucleoside cytidine. After intracellular phosphorylation to cytarabine triphosphate, it exerts its cytotoxic action by inhibiting the synthesis of DNA. It is the cornerstone drug of the treatment of acute myelogenous leukemia, being used during the different phases (induction, consolidation, maintenance) with various regimens and a wide dosage range (from 100 mg/m² to 6 g/m² per day) (5). Cytarabine is also active in the treatment of acute lymphoblastic leukemia and non-Hodgkin’s lymphomas. At high dose, cytarabine is given by venous infusion over 1-3 h. Regarding low doses (50-100 mg/m² per injection, *i.e.* low injection volumes), subcutaneous injection was seen as a practical alternative to the intravenous route for patients with acute myelogenous leukemia treated during the induction or re-induction phase.

Clinical pharmacokinetics of subcutaneous cytarabine are scarce (Table II). After bolus subcutaneous injection (100 mg/m²) in five adult patients with acute myelogenous leukemia, cytarabine was rapidly absorbed (time for plasma peak around 0.5 h based on the plasma concentration–time curve). Exposures (areas under the plasma concentration–time curve, or AUC) following subcutaneous and intravenous administrations were similar (absolute bioavailability: 100%) (6). Subcutaneous infusion (100 mg/m² over 12 h) has also been investigated in six patients with acute myelogenous leukemia providing comparable AUC when compared to the 12-h venous infusion (7). Currently, cytarabine is given subcutaneously by bolus injection in the induction (or re-induction) phase of acute myelogenous leukemia.

**Azacitidine.** Azacitidine is also an analog of cytidine approved as a single agent in the treatment of various hematological disorders (certain myelodysplastic syndromes, chronic myelomonocytic leukemia, acute myelogenous leukemia). After conversion to azacitidine triphosphate in tumour cells, azacitidine reverses aberrant DNA hypermethylation (as a so-called hypomethylating agent) resulting in re-expression of silenced genes and cell differentiation (8). In Europe,
azacitidine is only given subcutaneously at 75 mg/m² daily for seven days, every 28 days for at least six months of treatment, whereas in the USA, it can also be injected intravenously. Above 100 mg (4 ml), the administration necessitates two sites of subcutaneous injection by two syringes). According to the package insert, azacitidine is injected in the upper arm, the abdomen and the thigh, and the injection sites have to be alternated to optimize tolerance. The use of warm compresses after subcutaneous injection may reduce symptoms (8). An oral formulation of azacitidine is under development and might improve the ease of the treatment.

The absolute bioavailability was assessed in six patients with myelodysplastic syndromes who received azacitidine at 75 mg/m² as a single dose, subcutaneously and intravenously (9). Following subcutaneous injection, azacitidine was rapidly absorbed (time for plasma peak: 0.5 h). The plasma peak was higher after intravenous injection (2750 ng/ml versus 750 ng/ml following subcutaneous injection). Half-lives of both were short (<1 h) and the systemic clearances were very high (around 2.5 l/min). The absolute bioavailability was 89% (Table II). Another study evaluating the relative bioavailability of oral azacitidine in 42 patients with various hematological disorders found similar pharmacokinetic parameters relative to the subcutaneous injection (10).

Cladribine. Cladribine or 2-chlorodeoxy-2’d-adenosine is a purine analog used as single-agent therapy in patients with hairy cell leukemia, a very rare hematological disease. It is also a pro-drug that must be phosphorylated in leukemia cells in order to be cytotoxic (11). Cladribine may be administered by a single continuous venous infusion (at 0.1 mg/kg/day) over seven days. It is also available in a subcutaneous formulation under another trademark and is given as a single course of five daily subcutaneous injections of 0.14 mg/kg, representing a volume of injection of 5 ml for a 70-kg adult.

Initial pharmacokinetic determination indicated short plasma and intracellular half-lives supporting, in part, the use of continuous venous infusion. The re-appraisal of the pharmacokinetic profile of cladribine by high-performance liquid chromatography underlined a lower clearance and the possibility of intermittent administration (12). Therefore, subcutaneous injection was tested as a more convenient mode of administration. The kinetic profile was investigated in 10 patients with chronic lymphocytic leukemia or non-Hodgkin’s lymphoma who received cladribine (0.14 mg/kg) by intravenous and subcutaneous injections (Table II) (13). The absolute bioavailability was 102% and the concentration peak was higher after subcutaneous injection (318 nM) than after intravenous infusion (169 nM) due to the duration of the perfusion (2 h). Terminal half-lives were comparable (10-13 h) (13). Clinically, subcutaneous injection was similar to the continuous intravenous route and was characterized by the absence of local toxicity (11).

Bortezomib. Bortezomib is an atypical anticancer agent acting as an inhibitor of the proteasome, an enzyme complex involved in the degradation of cytotoxic proteins (14). Bortezomib is used alone or combined with other agents in the treatment of multiple myeloma (official labeling in Europe) and mantle cell lymphoma, with a cumbersome schedule of administration (1.3 mg/m² by bolus intravenous injection on days 1, 4, 8, 11 of a 21-day cycle, repeated for 6-8 cycles). The subcutaneous administration (same schedule) was tested as a more practical route and compared in terms of activity (overall response rate after four cycles) to the intravenous bolus in a non-inferiority randomized phase III trial including 222 patients with myeloma (15). Subcutaneous bortezomib was non-inferior to intravenous bortezomib (overall response rate: 42% in both groups). By contrast, patients treated subcutaneously displayed less peripheral neurotoxicity (all grades, 38% versus 53%; p=0.044). However, 6% of the patients had subcutaneous injection-site reactions (15). Subcutaneous bortezomib was approved in 2012 with the same 3.5 mg intravenous formulation (the powder is reconstituted with 1.4 ml of normal saline, instead of 3.5 ml, leading to a concentrated solution of 2.5 mg/ml).

Two comparative pharmacokinetic studies (n=24; n=31) have been performed showing comparable systemic exposures over 72 h (bioavailability 100%) with a little delay in absorption following subcutaneous injection (time for plasma peak: 0.5 h) (Table II) (15, 16). Terminal half-lives were comparable after intravenous and subcutaneous injection (around 65-95 h) (16). In addition, pharmacokinetics were not dependent on the site of subcutaneous injection (15). As for azacitidine, injection sites have to be alternated for tolerance reasons (thigh, abdomen) and it is recommended to use a new needle for injection (not that used to aspirate the solution in the vial). A retrospective study in 15 Japanese patients with myeloma has suggested a better local tolerance after injection in the abdomen than in the thigh (17). Moderate-to-severe injection-site reactions (grade 2) occurred in five patients primarily after administration in the thigh. Overall, the occurrence of grade 2 site reactions was lower after abdominal injections (1.9%; 1.1%) than after thigh injections (6/65; 9.2%) (17). A single-center study including 47 patients with myeloma reported that 68% of patients preferred the subcutaneous administration (18). Subcutaneous bortezomib alone or combined with oral anti-myeloma agents (immunomodulatory drugs such as thalidomide and analogs, corticosteroids) thus enhance the convenience of treatment for patients with myeloma.

Omacetaxine. Omacetaxine (mepesuccinate) is the semi-synthetic form of homoharringtonine, an alkaloid extracted from Cephalotaxus species that has shown activity in the treatment of chronic myeloid leukemia (19). Omacetaxine
inhibits protein synthesis and is currently approved in the USA for the treatment of adult patients with chronic or accelerated-phase chronic myeloid leukemia with resistance or intolerance to two or more tyrosine kinase inhibitors. Omacetaxine is available (at least in France) through a compassionate-use program. The treatment of chronic myeloid leukemia is primarily ambulatory and includes five oral kinase inhibitors (imatinib, dasatinib, nilotinib, bosutinib, ponatinib). Omacetaxine (1.25 \text{mg/cm}^2) is given subcutaneously twice-a-day for two weeks (induction) or for one week (maintenance) every month. Based on data from two phase II studies, median exposure to omacetaxine is 7.5 months (range: 0.03-38.6 months) (20). The subcutaneous route was chosen as a continuous low-dose schedule to limit the cardiovascular side-effects of short intravenous injections of homoharringtonine (19). From a pragmatic point of view, subcutaneous administration must be performed by healthcare professionals (no self-administration) and in our experience, patients are hospitalized during the cycle. Although less convenient than oral tyrosine kinase inhibitors, this modality of administration also constitutes an alternative treatment for non-adherent patients.

Pharmacokinetics of subcutaneous omacetaxine (1.25 \text{mg/cm}^2 twice daily) have been studied on day 1 and 11 during a 2-week cycle in 21 adult patients with various types of cancer (Table II) (21). Pharmacokinetic profiles were comparable at day 1 and day 11, except for the plasma peak that was greater at day 11 (36.4 \text{ng/ml} \textit{versus} 25.1 \text{ng/ml} at day 1). Absorption was rapid (time for plasma peak: 0.55 h) and the terminal half-life was 7 h. The bioavailability of omacetaxine is unknown but is estimated to be around 70-90\% based on pharmacokinetics of intravenous homoharringtonine (21).

\textit{Bleomycin}. Bleomycin is a major anticancer agent used in combination, in the treatment of germ cell testicular cancer and lymphomas. Bleomycin acts by cleaving DNA and is primarily given by short intravenous injection (22). It can also be administered by venous infusion, intramuscular injection and subcutaneous infusion (package insert). The use of bleomycin given by continuous subcutaneous infusion is little documented (23, 24). A pharmacokinetic study comparing subcutaneous and venous infusion over 24 h in nine patients with various types of cancer showed comparable exposures (bioavailability around 90\%) (Table II) (23). In clinical practice, subcutaneous infusion of bleomycin is anecdotal.

\textit{Monoclonal antibodies}. Monoclonal antibodies are a therapeutic source used in the management of various diseases. Due to their very long half-life (2-3 weeks), they are given intermittently, either by intravenous perfusion (requiring short hospitalization to manage potential infusion-related reactions), or by subcutaneous injection (ambulatory treatment and possible self-administration at home). Subcutaneous administration of monoclonal antibodies is already performed, for example, in the treatment of rheumatoid arthritis or Crohn’s disease with adalimumab, certolizumab pegol and golimumab, and in the treatment of asthma with omalizumab. The mechanisms of absorption of antibodies following subcutaneous administration has been reviewed in depth (25, 26). After subcutaneous injection, monoclonal antibodies appear to reach the blood through the lymphatics by the intermediate of the neonatal receptor (FcRn) also involved in the transport across epithelial cells and the elimination of immunoglobulins (25-27).

In oncology, monoclonal antibodies have been used since 1997 (rituximab) (28). They are given in the hospital by venous perfusions over 0.5-4 h, mostly at dosages based on body size. In 2013, a subcutaneous formulation of trastuzumab was approved in Europe and will be followed by rituximab in the near future.

\textit{Alemtuzumab}. First attempts at subcutaneous delivery of monoclonal antibodies in oncology were carried out with alemtuzumab, an antibody that targets lymphocytes through their membrane antigen CD52 (29). Alemtuzumab (also known as campath-1H) has been investigated for a long time (since the end of the 1980s) as a lympholytic agent in autoimmune diseases, transplantation and cancer, both intravenously and subcutaneously. Alemtuzumab was finally approved (2001) as single agent in the treatment of chronic lymphocytic leukemia given intravenously at a fixed dose of 30 mg, thrice a week, for up to 12 weeks. The schedule was set empirically and unfortunately does not refer to the long half-life of the antibody (around three weeks) (27). Rather than investigating less frequent administrations (by analogy to other antibodies), optimization of the schedule went through the subcutaneous delivery. Two phase II trials (30 mg thrice a week, subcutaneously) either in untreated (n=41) or in pretreated patients with lymphocytic leukemia (n=103) showed activity at least comparable to that of the intravenous (approved) route in pretreated patients (overall response rate: 34\%; median overall survival: 19.1 months) (29, 30). No randomized trial has compared the two modes of administration. In addition, self-administration by the patient was judged feasible (29, 31). Pharmacokinetics of subcutaneous alemtuzumab are little documented and the absolute bioavailability is unknown (Table II). Given the slow elimination, pharmacokinetic documentation should necessitate the administration of a single injection with a very long sampling duration to characterize the terminal phase of elimination. The current schedule of administration (three injections per week) precludes any kinetic study. Hale \textit{et al.} compared blood trough concentrations from patients treated either intravenously or subcutaneously (30 mg thrice weekly) (32). Maximal trough concentrations were similar.
formulation, subcutaneous trastuzumab is given at a fixed dose (600 mg every three weeks) without a loading dose. Regarding intravenous trastuzumab, it has to be said that neither body weight-based dosing nor the use of a loading dose have proven to be of clinical significance (33).

Intravenous and subcutaneous formulations have been compared in a non-inferiority multi-center randomized trial that included 596 women with HER2-positive early breast cancer (35). Trastuzumab was given every three weeks either intravenously (8 mg/kg then 6 mg/kg according to the current labeling) or subcutaneously (fixed dose of 600 mg injected into the thigh by a nurse over 5 min) for eight cycles before surgery and for 10 cycles after surgery (total duration of treatment: one year). Efficacy, safety and pharmacokinetics were assessed. The subcutaneous formulation was non-inferior with respect to the proportion of patients with antitumoral response (pathological complete response) evaluated before surgery (intravenous: 40.7%; subcutaneous: 45.4%) and to the geometric mean trough blood concentrations measured after seven cycles (intravenous: 51.8 μg/ml; subcutaneous: 69 μg/ml). However, when compared to the intravenous route, more patients had serious side-effects in the subcutaneously-treated group (21% versus 12%), particularly infections and infestations (8.1% versus 4.4%). Regarding local tolerance, 11.1% of the patients had injection-site reactions (mostly grade 1) following subcutaneous administration (35). Another randomized trial assessed patient preference for either subcutaneous or intravenous injection of trastuzumab (36). Patients with early breast cancer received both formulations from a healthcare professional in the clinic (no self-administration at home). Not surprisingly, among the 236 evaluable patients, 91.5% preferred subcutaneous injection. The two main reasons were time-saving (due to the very short duration of subcutaneous injection) and less pain and discomfort (36).

More detailed pharmacokinetic data have been obtained in healthy male volunteers (to avoid exposing healthy females and the risk of developing anti-trastuzumab antibodies that might affect any future treatment) and patients with HER2-positive breast cancer who received either the subcutaneous or the intravenous formulation as a single dose (Table II) (37). Trastuzumab was given at a dose based on body weight varying between 6 and 12 mg/kg. Following subcutaneous administration, trastuzumab was slowly absorbed (time for plasma peak: 4-6 days). Regarding the therapeutic dose (6 mg/kg), the absolute bioavailability of the subcutaneous formulation was 84% (Table II) (37). So, when considering for other monoclonal antibodies (absolute bioavailability around 50-80%), trastuzumab formulated with hyaluronidase appears to be well-absorbed after subcutaneous injection (2, 26). The terminal half-lives were similar after intravenous and subcutaneous injection (10 days), inferior to the 28.5 days previously reported with the intravenous formulation due to a different compartmental kinetic analysis (37).

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**Trastuzumab.** Trastuzumab is a monoclonal antibody that targets the transmembrane receptor HER2 (Human Epidermal growth factor Receptor 2) overexpressed in certain types of cancer (also called HER2-positive) and associated with poor prognosis without specific (anti-HER2) treatment. Antitumoral activity derives from several mechanisms that include antibody dependent cellular cytotoxicity, inhibition of ectodomain cleavage, induction of apoptosis (33). Trastuzumab is approved for the treatment of breast cancer overexpressing HER2 (15% of breast cancer) and the treatment of metastatic gastric cancer overexpressing HER2 (around 20% of gastric cancer) (33, 34). Trastuzumab is given intravenously (1 h 30 min for the first dose and 30 min for subsequent perfusions) weekly or every three weeks, at a dose adjusted on body weight that includes a loading dose at the first administration. The duration of treatment is set at one year in early breast cancer (before and after surgery) and is variable in metastatic disease (until unsatisfactory response, unacceptable toxicity or patient’s wish) but can last over 10 years in certain patients with breast cancer. Trastuzumab is given with other anticancer agents, or alone (late-phase treatment of early breast cancer).

A more convenient way to use trastuzumab was to develop subcutaneous administration. Contrasting with alemtuzumab, which is injected at a low dose (30 mg), trastuzumab is administered at doses ranging between 100-600 mg, according to body weight and the frequency of injection (weekly or tri-weekly). Using the intravenous formulation, theses weekly or tri-weekly dosages represent volumes of 5-30 ml of reconstituted solution that impede subcutaneous delivery. A subcutaneous and concentrated formulation set at a fixed dose of 600 mg (120 mg/ml) was developed integrating recombinant human hyaluronidase (10000 UI), an enzyme that temporarily degrades the extracellular matrix and facilitates absorption. This formulation has been approved in Europe (September 2013) and is dedicated to the treatment of breast cancer (i.e. not approved for metastatic gastric cancer). When compared to the intravenous formulation, subcutaneous trastuzumab is given at a fixed dose (600 mg every three weeks) without a loading dose. Regarding intravenous trastuzumab, it has to be said that neither body weight-based dosing nor the use of a loading dose have proven to be of clinical significance (33).
Pharmacokinetics of subcutaneous trastuzumab given by a single-use injection device enabling self-administration (not approved) and by a syringe have been compared in 119 randomized healthy male volunteers (38). Pharmacokinetic parameters were similar between the two modalities of subcutaneous administration (Table II).

Results from a prospective, two-cohort, non-randomized trial (SafeHer, NCT01566721) are awaited. This study is testing safety and tolerability of subcutaneous trastuzumab (600 mg every three weeks for one year) in patients with early breast cancer via assisted administration with the vial formulation or self-administration with a ready to use subcutaneous injection device.

Conclusion

All anticancer agents given subcutaneously display a very high bioavailability and are rapidly absorbed except the monoclonal antibodies trastuzumab and alemtuzumab. Subcutaneous delivery does not impact the rate of elimination when compared to the intravenous route (azacitidine, cladribine, bortezomib, trastuzumab). Some formulations may be self-administered in educated patients (methotrexate, cladribine) but others require hospitalization (omacetaxine). Although less convenient than the oral route, subcutaneous delivery constitutes an alternative of treatment for non-adherent patients with chronic myelogenous leukaemia (omacetaxine). The anatomical regions of administration need to be alternated for tolerance reasons (azacitidine, bortezomib) and do not have any impact on absorption (bortezomib). When available, comparative studies with intravenous administration showed comparable clinical issues, with an advantage for subcutaneous bortezomib regarding the occurrence of peripheral neurotoxicity. In treatment of myeloma, subcutaneous bortezomib combined with oral agents enhances the convenience of treatment (limits hospitalization). Subcutaneous formulations of trastuzumab and, in the near future rituximab, are easier to use (fixed dose instead of body size-based, absence of loading dose, very short duration of injection). They might allow for ambulatory treatment and self-administration (of interest if the patient is not concurrently receiving intravenous therapies). From an economic point of view, subcutaneous formulations of monoclonal antibodies may reduce healthcare costs but will have to face the arrival of less expensive intravenous biologically-similar agents (‘biosimilars’) that will reduce the cost of hospitalization.

References


